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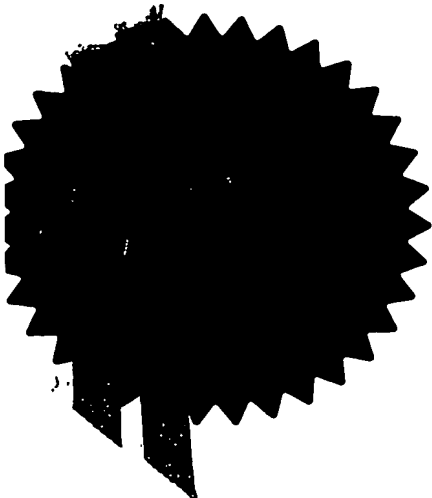Newport
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

CAMBRIDGE ANTIBODY TECHNOLOGY LIMITED
Milstein Building
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Cambridge CB1 6GH

Patents ADP number *(if you know it)*   5781992003

If the applicant is a corporate body, give the country/state of its incorporation

GB

4. Title of the invention

HUMAN ANTIBODY MOLECULES

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

MEWBURN ELLIS
York House
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London WC2B 6HP

Patents ADP number *(if you know it)*   ~~109006~~   8836884001

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	US	60/487,512	15 JULY 2003

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Continuation sheets of this form	0
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11. I/We request the grant of a patent on the basis of this application.

Signature(s)   *[signature]*   Date 29 MARCH 2004

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HUMAN ANTIBODY MOLECULES

The present invention relates to specific binding members, in particular human anti-IL-13 antibody molecules and especially those which neutralise IL-13 activity. It further relates to methods for using anti-IL-13 antibody molecules in diagnosis or treatment of IL-13 related disorders, including asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

Preferred embodiments of the present invention employ the antibody VH and/or VL domain of the antibody molecule herein termed BAK502G9 and other antibody molecules of the BAK502G9 lineage and of the BAK278D6 lineage, as herein defined. Further preferred embodiments employ complementarity determining regions (CDRs) of the BAK278D6 lineage, and preferably BAK502G9, especially VH CDR3 in other antibody framework regions. Further aspects of the present invention provide for compositions containing specific binding members of the invention, and their use in methods of inhibiting or neutralising IL-13, including methods of treatment of the human or animal body by therapy.

The present invention provides antibody molecules of particular value in binding and neutralising IL-13, and thus of use in any of a variety of therapeutic treatments, as indicated by the experimentation contained herein and further by the supporting technical literature.

Interleukin (IL)-13 is a 114 amino acid cytokine with an unmodified molecular mass of approximately 12 kDa [1,2]. IL-13 is most closely related to IL-4 with which it shares 30% sequence similarity at the amino acid level. The human IL-13 gene is located on chromosome 5q31 adjacent to the IL-4 gene

[1][2]. This region of chromosome 5q contains gene sequences for other Th2 lymphocyte derived cytokines including GM-CSF and IL-5, whose levels together with IL-4 have been shown to correlate with disease severity in asthmatics and rodent

5   models of allergic inflammation [3][4][5][6][7][8].

Although initially identified as a Th2 CD4+ lymphocyte derived cytokine, IL-13 is also produced by Th1 CD4+ T-cells, CD8+ T lymphocytes NK cells, and non-T-cell populations such as mast

10 cells, basophils, eosinophils, macrophages, monocytes and airway smooth muscle cells.

IL-13 is reported to mediate its effects through a receptor system that includes the IL-4 receptor α chain (IL-4Rα), which

15 itself can bind IL-4 but not IL-13, and at least two other cell surface proteins, IL-13Rα1 and IL-13Rα2 [9][10]. IL-13Rα1 can bind IL-13 with low affinity, subsequently recruiting IL-4Rα to form a high affinity functional receptor that signals [11][12]. The Genbank database lists the amino acid sequence

20 and the nucleic acid sequence of IL-13Rα1 as NP_001551 and Y10659 respectively. Studies in STAT6 (signal transducer and activator of transcription 6) -deficient mice have revealed that IL-13, in a manner similar to IL-4, signals by utilising the JAK-STAT6 pathway [13][14].   IL-13Rα2 shares 37% sequence

25 identity with IL-13Rα1 at the amino acid level and binds IL-13 with high affinity [15][16]. However, IL-13Rα2 has a shorter cytoplasmic tail that lacks known signalling motifs. Cells expressing IL-13Rα2 are not responsive to IL-13 even in the presence of IL-4Rα [17]. It is postulated, therefore, that IL-

30 13Rα2 acts as a decoy receptor regulating IL-13 but not IL-4 function. This is supported by studies in IL-13Rα2 deficient mice whose phenotype was consistent with increased responsiveness to IL-13 [18][19]. The Genbank database lists

the amino acid sequence and the nucleic acid sequence of IL-13Rα2 as NP_000631 and Y08768 respectively.

The signalling IL-13Rα1/IL-4Rα receptor complex is expressed
5   on human B-cells, mast cells, monocyte/macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, airway epithelial cells and airway smooth muscle cells.

Bronchial asthma is a common persistent inflammatory disease
10   of the lung characterised by airways hyper-responsiveness, mucus overproduction, fibrosis and raised serum IgE levels. Airways hyper-responsiveness (AHR) is the exaggerated constriction of the airways to non-specific stimuli such as cold air. Both AHR and mucus overproduction are thought to be
15   responsible for the variable airway obstruction that leads to the shortness of breath characteristic of asthma attacks (exacerbations) and which is responsible for the mortality associated with this disease (around 2000 deaths/year in the United Kingdom).
20

The incidence of asthma, along with other allergic diseases, has increased significantly in recent years [20][21]. For example, currently, around 10% of the population of the United Kingdom (UK) has been diagnosed as asthmatic.
25

Current British Thoracic Society (BTS) and Global Initiative for Asthma (GINA) guidelines suggest a stepwise approach to the treatment of asthma [22, 23]. Mild to moderate asthma can usually be controlled by the use of inhaled corticosteroids,
30   in combination with beta-agonists or leukotriene inhibitors. However, due to the documented side effects of corticosteroids, patients tend not to comply with the treatment regime which reduces the effectiveness of treatment [24-26].

There is a clear need for new treatments for subjects with more severe disease, who often gain very limited benefit from either higher doses of inhaled or oral corticosteroids

5   recommended by asthma guidelines. Long term treatment with oral corticosteroids is associated with side effects such as osteoporosis, slowed growth rates in children, diabetes and oral candiditis [88]. As both beneficial and adverse effects of corticosteroids are mediated via the same receptor,

10   treatment is a balance between safety and efficacy. Hospitalisation of these patients, who represent around 6% of the UK asthma population, as a result of severe exacerbations accounts for the majority of the significant economic burden of asthma on healthcare authorities [89].

15

It is believed that the pathology of asthma is caused by ongoing Th2 lymphocyte mediated inflammation that results from inappropriate responses of the immune system to harmless antigens. Evidence has been accrued which implicates IL-13,

20   rather than the classical Th2 derived cytokine IL-4, as the key mediator in the pathogenesis of established airway disease.

Administration of recombinant IL-13 to the airways of naïve

25   non-sensitised rodents caused many aspects of the asthma phenotype including airway inflammation, mucus production and AHR [27][28][29][30]. A similar phenotype was observed in a transgenic mouse in which IL-13 was specifically overexpressed in the lung. In this model more chronic exposure to IL-13 also

30   resulted in fibrosis [31].

Further, in rodent models of allergic disease many aspects of the asthma phenotype have been associated with IL-13. Soluble murine IL-13Rα2, a potent IL-13 neutraliser, has been shown to

inhibit AHR, mucus hypersecretion and the influx of inflammatory cells which are characteristics of this rodent model [27][28][30]. In complementary studies, mice in which the IL-13 gene had been deleted, failed to develop allergen-

5 induced AHR. AHR could be restored in these IL-13 deficient mice by the administration of recombinant IL-13. In contrast, IL-4 deficient mice developed airway disease in this model [32][33].

10 Using a longer-term allergen-induced pulmonary inflammation model, Taube at al. demonstrated the efficacy of soluble murine IL-13Rα2 against established airway disease [34]. Soluble murine IL-13Rα2 inhibited AHR, mucus overproduction and to a lesser extent airway inflammation. In contrast,

15 soluble IL-4Rα, which binds and antagonises IL-4, had little effect on AHR or airway inflammation in this system [35]. These findings were supported by Blease et al. who developed a chronic fungal model of asthma in which polyclonal antibodies against IL-13 but not IL-4 were able to reduce mucus

20 overproduction, AHR and subepithelial fibrosis [36].

A number of genetic polymorphisms in the IL-13 gene have also been linked to allergic disease. In particular, a variant of the IL-13 gene in which the arginine residue at amino acid 130

25 is substituted with glutamine (Q130R) has been associated with bronchial asthma, atopic dermatitis and raised serum IgE levels [37][38][39][40]. This particular IL-13 variant is also referred to as the Q110R variant (arginine residue at amino acid 110 is substituted with glutamine) by some groups who

30 exclude the 20 amino acid signal sequence from the amino acid count. Arima et al, [41] report that this variant is associated with raised levels of IL-13 in serum. The IL-13 variant (Q130R) and antibodies to this variant are discussed in WO 01/62933. An IL-13 promoter polymorphism, which alters

IL-13 production, has also been associated with allergic asthma [42].

Raised levels of IL-13 have also been measured in human
5 subjects with asthma, atopic rhinitis (hay fever), allergic dermatitis (eczema) and chronic sinusitis. For example levels of IL-13 were found to be higher in bronchial biopsies, sputum and broncho-alveolar lavage (BAL) cells from asthmatics compared to control subjects [43][44][45][46]. Further, levels
10 of IL-13 in BAL samples increased in asthmatic individuals upon challenge with allergen [47][48]. The IL-13 production capacity of CD4(+) T cells has further been shown to be useful marker of risk for subsequent development of allergic disease in newborns [49].
15

Li et al [114] have recently reported affects of a neutralising anti-mouse IL-13 antibody in a chronic mouse model of asthma. Chronic asthma-like response (such as AHR, sever airway inflammation, hyper mucus productions) was
20 induced in OVA sensitised mice. Li et al report that administration of an IL-13 antibody at the time of each OVA challenge suppresses AHR, eosinophil infiltration, serum IgE levels, proinflammatory cytokine/chemokine levels and airway remodelling [14].
25

IL-13 may play a role in the pathogenesis of inflammatory bowel disease. Heller et al.[116] report that neutralisation of IL-13 by administration of soluble IL-13Rα2 ameliorated colonic inflammation in a murine model of human ulcerative
30 colitis [116]. Correspondingly, IL-13 expression was higher in rectal biopsy specimens from ulcerative colitis patients when compared to controls [117].

In summary, these data provide indication that IL-13 rather than IL-4 is a more attractive target for the treatment of human allergic disease.

5   Aside from asthma, IL-13 has been associated with other fibrotic conditions. Increased levels of IL-13, up to a 1000 fold higher than IL-4, have been measured in the serum of patients with systemic sclerosis [50] and in BAL samples from patients affected with other forms of pulmonary fibrosis [51].

10   Correspondingly, overexpression of IL-13 but not IL-4 in the mouse lung resulted in pronounced fibrosis [52][53]. The contribution of IL-13 to fibrosis in tissues other than the lung has been extensively studied in a mouse model of parasite-induced liver fibrosis. Specific inhibition of IL-13

15   by administration of soluble IL-13Rα2 or IL-13 gene disruption, but not ablation of IL-4 production prevented fibrogenesis in the liver [54][55][56].

Chronic Obstructive Pulmonary Disease (COPD) includes patient

20   populations with varying degrees of chronic bronchitis, small airway disease and emphysema and is characterised by progressive irreversible lung function decline that responds poorly to current asthma based therapy [90].
The incidence of COPD has risen dramatically in recent years

25   to become the fourth leading cause of death worldwide (World Health Organisation). COPD therefore represents a large unmet medical need.

The underlying causes of COPD remain poorly understood. The

30   "Dutch hypothesis" proposes that there is a common susceptibility to COPD and asthma and therefore, that similar mechanisms may contribute to the pathogenesis of both disorders [57].

Zheng et al [58] have demonstrated that overexpression of IL-13 in the mouse lung caused emphysema, elevated mucus production and inflammation, reflecting aspects of human COPD. Furthermore, AHR, an IL-13 dependent response in murine models

5 of allergic inflammation, has been shown to be predictive of lung function decline in smokers [59]. A link has also been established between an IL-13 promoter polymorphism and susceptibility to develop COPD [60].

10 The signs are therefore that IL-13 plays an important role in the pathogenesis of COPD, particularly in patients with asthma-like features including AHR and eosinophilia. mRNA levels of IL-13 have been shown to be higher in autopsy tissue samples from subjects with a history of COPD when compared to

15 lung samples from subjects with no reported lung disease (J. Elias, Oral communication at American Thoracic Society Annual Meeting 2002). In another study, raised levels of IL-13 were demonstrated by immunohistochemistry in peripheral lung sections from COPD patients [91].

20

Hodgkin's disease is a common type of lymphoma, which accounts for approximately 7,500 cases per year in the United States. Hodgkin's disease is unusual among malignancies in that the neoplastic Reed-Sternberg cell, often derived from B-cells,

25 make up only a small proportion of the clinically detectable mass. Hodgkin's disease-derived cell lines and primary Reed-Sternberg cells frequently express IL-13 and its receptor [61]. As IL-13 promotes cell survival and proliferation in normal B-cells, it was proposed that IL-13 could act as a

30 growth factor for Reed-Sternberg cells. Skinnider et al. have demonstrated that neutralising antibodies against IL-13 can inhibit the growth of Hodgkin's disease-derived cell lines *in vitro* [62]. This finding suggested that Reed-Sternberg cells might enhance their own survival by an IL-13 autocrine and

paracrine cytokine loop. Consistent with this hypothesis, raised levels of IL-13 have been detected in the serum of some Hodgkin's disease patients when compared to normal controls [63]. IL-13 inhibitors may therefore prevent disease

5 progression by inhibiting proliferation of malignant Reed-Sternberg cells.

Many human cancer cells express immunogenic tumour specific antigens. However, although many tumours spontaneously

10 regress, a number evade the immune system (immunosurveillance) by suppressing T-cell mediated immunity. Terabe et al.[64] have demonstrated a role of IL-13 in immunosuppression in a mouse model in which tumours spontaneously regress after initial growth and then recur. Specific inhibition of IL-13,

15 with soluble IL-13Rα2, protected these mice from tumour recurrence. Terabe et al [64] went on to show that IL-13 suppresses the differentiation of tumour specific CD8+ cytotoxic lymphocytes that mediate anti-tumour immune responses.

20

IL-13 inhibitors may, therefore, be used therapeutically to prevent tumour recurrence or metastasis. Inhibition of IL-13 has been shown to enhance anti-viral vaccines in animal models and may be beneficial in the treatment of HIV and other

25 infectious diseases [65].

It should be noted that generally herein reference to interleukin-13 or IL-13 is, except where context dictates otherwise, reference to human IL-13. This is also referred to

30 in places as "the antigen". The present invention provides antibodies to human IL-13, especially human antibodies, that are cross-reactive with non-human primate IL-13, including cynomolgus and rhesus monkey IL-13. Antibodies in accordance with some embodiments of the present invention recognise a

variant of IL-13 in which the arginine residue at amino acid position 130 is replaced by glutamine. In other aspects and embodiments the present invention provides specific binding members against murine IL-13, specifically mouse IL-13.

5

Brief Description of Figures

Figure 1 shows neutralisation potency (% inhibition) of BAK167A11 (closed squares) and its derivative BAK615E3 (open
10   squares) as scFv against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay. The triangles represent irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

15 . Figure 2 shows the neutralisation potency (% inhibition) of BAK278D6 (closed squares) and its derivative BAK502G9 (open squares) as scFv against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay. The triangles represent irrelevant scFv. Data represent the mean with standard error bars of triplicate
20   determinations within the same experiment.

Figure 3 shows the neutralisation potency (% inhibition) of BAK209B11 (closed squares) as a scFv against 25 ng/ml murine IL-13 in the TF-1 cell proliferation assay. The triangles.
25   represent irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 4 shows the neutralisation potency (% inhibition) of
30   BAK278D6 (closed squares) as a scFv against IL-13 in the TF-1 cell proliferation assay. The triangles represent irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.
        Figure 4A show potency against 25 ng/ml human IL-13.

Figure 4B shows potency against 25 ng/ml human variant IL-13.

Figure 4C shows potency against 50 ng/ml non-human primate IL-13.

5

Figure 5 shows a comparison of the potency of anti-human IL-13 antibodies in the TF-1 proliferation assay. Data represent the mean neutralisation potency with standard error bars over 5-7 experiments against 25 ng/ml human IL-13. The performance

10   relative to the commercially available antibody, B-B13, was evaluated statistically by performing a one-way ANOVA with Dunnett's test. * P<0.05, ** P<0.01 compared to B-B13.

Figure 6 shows the neutralisation potency (% inhibition) of

15   BAK502G9 (closed squares), BAK1167F2 (closed triangles) and BAK1183H4 (closed inverted triangles) as human IgG4 against tagged IL-13 in the TF-1 cell proliferation assay. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of three separate experiments.

20   Figure 6A shows potency against 25 ng/ml human IL-13.

Figure 6B shows potency against 25 ng/ml human variant IL-13.

Figure 6C shows potency against 50 ng/ml non-human primate IL-13.

25

Figure 7 shows the neutralisation potency (% inhibition) of BAK502G9 (closed squares), BAK1167F2 (closed triangles), BAK1183H4 (closed inverted triangles) as human IgG4 and commercial anti-human IL-13 antibodies (B-B13 - open squares;

30   JES10-5A2 - open inverted triangles) in the native IL-13 dependent HDLM-2 cell proliferation assay. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 8 shows a comparison of the potency of anti-human IL-13 antibodies in the NHLF assay. Data represent the mean neutralisation potency (IC_{50} pM) with standard error bars over

5 4-5 experiments against 10 ng/ml human IL-13 in the NHLF eotaxin release assay. The performance relative to the commercially available antibody, B-B13, was evaluated statistically by performing a one-way ANOVA with Dunnett's test. * $P<0.05$, ** $P<0.01$ compared to B-B13.

10

Figure 9 shows the neutralisation potency (% inhibition) of BAK502G9 (closed triangles), BAK1167F2 (closed inverted triangles), BAK1183H4 (diamonds) as human IgG4 against VCAM-1 upregulation on the surface of HUVEC in response to 10ng/ml

15 human IL-13. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 10 shows the neutralisation potency (% inhibition) of

20 BAK502G9 (closed squares), BAK1167F2 (closed triangles), BAK1183H4 (closed inverted triangles) as human IgG4 against eotaxin release from VCAM-1 upregulation on the surface of HUVEC in response to either 1ng/ml human IL-4 (Figure 10A) or 0.5ng/ml human IL-1β (Figure 10B). Open triangles represent

25 irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 11 shows the neutralisation potency (% inhibition) of BAK209B11 (squares) as a human IgG4 against 1 ng/ml murine IL-

30 13 in the factor dependent B9 cell proliferation assay. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 12 shows the relative level of IL-13 in lung homogenates from sensitised (s)(right-hand bar) and non-sensitised (ns)(left-hand bar) mice post challenge in a murine model of acute pulmonary allergic inflammation. The effect of

5   sensitisation was statistically evaluated by performing Student's t-test using quantity of IL-13 data. *<0.05. **<0.01 compared to non-sensitised control animals (n=5-6 mice). Data represent the mean with standard error bars.

10   Figure 13 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin induced leukocyte recruitment to the lung in ovalbumin sensitised mice. The number of leukocytes is shown (x 10^4).

15   The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. *<0.05. **<0.01 compared to ovalbumin challenged PBS control animals (=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

20

Figure 14 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin induced eosinophil recruitment to the lung in ovalbumin

25   sensitised mice. The number of eosinophils is shown (x 10^4). The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. *<0.05. **<0.01 compared to ovalbumin challenged PBS control animals (=0% inhibition; n=5-

30   8 mice). Data represent the mean with standard error bars.

Figure 15 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin

induced neutrophil recruitment to the lung in ovalbumin
sensitised mice. The number of neutrophils is shown (x 10^4).
The effect of antibody treatment was statistically evaluated
by performing one way ANOVA with Dunnett's test using

5   differential cell count data. *<0.05. **<0.01 compared to
ovalbumin challenged PBS control animals (=0% inhibition; n=5-
8 mice). Data represent the mean with standard error bars.

Figure 16 illustrates the effects of i.v. administration of

10 BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin
induced lymphocyte recruitment to the lung in ovalbumin
sensitised mice. The induction of lymphocytes was dose
dependently inhibited by BAK209B11 with maximal inhibition at

15 3µg/ml of BAK209B11. The effect of antibody treatment was
statistically evaluated by performing one way ANOVA with
Dunnett's test using differential cell count data. *<0.05.
**<0.01 compared to ovalbumin challenged PBS control animals
(=0% inhibition; n=5-8 mice). Data represent the mean with

20 standard error bars.

Figure 17 illustrates the effects of i.v. administration of
BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin

25 induced monocyte/macrophage recruitment to the lung in
ovalbumin sensitised mice. There was no significant increase
in the levels of monocytes/macrophages of sensitised animals
when compared with control animals. However, such background
levels of these cells were depressed by ≥36µg/ml BAK209B11 in

30 sensitised animals. The effect of antibody treatment was
statistically evaluated by performing one way ANOVA with
Dunnett's test using differential cell count data. *<0.05.
**<0.01 compared to ovalbumin challenged PBS control animals

(=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

5   Figure 18 shows the effects of a commercial anti-IL-13 neutralising antibody JES10-5A2 on the influx of cells (number of leukocytes is shown (x 10^4)) into the murine airpouch elicited by administration of bacterially derived recombinant human IL-13. The effect of antibody treatment was
10   statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. *<0.05. **<0.01 compared to CMC control animals (=0% inhibition; n=11- 13 mice). Data represent the mean with standard error bars.

15   Figure 19 shows an sequence alignment of cynomolgus IL-13 against human IL-13 amino acid sequences. The seven amino acid residues that differ between human and cynomolgus IL-13 are shaded. Rhesus and cynomolgus IL-13 have an identical amino acid sequence.
20

Figure 20 illustrates the effects of single 10mg/kg i.v bolus dose of BAK502G9 as human IgG4 on serum IgE levels in 4 allergic but non-challenged cynomolgus primates (2 male/2 female) over 29 days. Serum IgE concentration is significantly
25   reduced from 100 % (predose) to 66 ± 10% of control values (p<0.05), at 4 and 5 days after dosing. This lowering of serum IgE concentration recovers to 88 ± 8 % of control levels by day 22. * = p<0.05 as compared to predose IgE levels, repeated measures ANOVA followed by Dunnett's multiple
30   comparisons test (n=4 animals).

Figure 21 illustrates the effects of intraperitoneal administration of BAK209B11 in different amounts (H=237μg/day, M=23.7μg/day and L=2.37μg/day) compared with an isotype

matched IgG1 irrelevant control antibody on the lung function of ovalbumin sensitised and challenged mice. In Figure 21A lung function is represented by log $PC_{50}s$ (log methacholine concentration required to increase baseline PenH by 50%)

5 before any treatment (day 0) and post sensitisation, challenge and drug treatment (day 25). Figure 21A shows the raw data used to calculate the study endpoint, shown in figure 21B (Delta log PC_{50}). Data represent the mean with standard error bars of n=8.

10

In Figure 21B changing lung function is shown by a change in an individual mouse's log PC_{50} (delta log PC_{50}). Delta log PC_{50} is defined as an individuals change in log PC_{50} at day 25 verus day 0. Data represent group mean delta log PC_{50} (individual

15 changes averaged within treatment groups) with standard error bars. The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using delta log PC_{50} data. **$p<0.01$ compared to ovalbumin sensitised and challenged control animals (n=8 mice).

20

Figure 22 illustrates the effects of local (i.po.) and systemic (i.v.) administration of BAK502G9 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on the total leukocyte recruitment

25 (Figure 22A) and eosinophil recruitment (Figure 22B) into the air pouch of BALB/C mice. Data represent the mean with standard error bars of n=10. The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using log-transformed data. *$p<0.05$, **$p<0.01$

30 compared to huIL-13 challenged mice (n=10).

In various aspects and embodiments of the invention there is provided the subject-matter of the claims included below.

The present invention provides specific binding members for
IL-13, in particular human and/or primate IL-13 and/or variant
IL-13 (Q130R), and murine IL-13. Preferred embodiments within
5   the present invention are antibody molecules, whether whole
antibody (e.g. IgG, such as IgG4) or antibody fragments (e.g.
scFv, Fab, dAb). Antibody antigen binding regions are
provided, as are antibody VH and VL domains. Within VH and VL
domains are provided complementarity determining regions,
10   CDR's, which may be provided within different framework
regions, FR's, to form VH or VL domains as the case may be.
An antigen binding site may consist of an antibody VH domain
and/or a VL domain.

15   An antigen binding site may be provided by means of
arrangement of CDR's on non-antibody protein scaffolds such as
fibronectin or cytochrome B etc. [115, 116]. Scaffolds for
engineering novel binding sites in proteins have been reviewed
in detail by Nygren et al [116]. Protein scaffolds for
20   antibody mimics are disclosed in WO/0034784 in which the
inventors describe proteins (antibody mimics) which include a
fibronectin type III domain having at least one randomised
loop. A suitable scaffold into which to graft one or more
CDR's, e.g. a set of HCDR's, may be provided by any domain
25   member of the immunoglobulin gene superfamily.

Preferred embodiments of the present invention are in what is
termed herein the "BAK278D6 lineage". This is defined with
reference to a set of six CDR sequences of BAK278D6 as
30   follows: HCDR1 (SEQ ID NO: 1), HCDR2 (SEQ ID NO: 2), HCDR3
(SEQ ID NO: 3), LCDR1 (SEQ ID NO: 4), LCDR2 (SEQ ID NO: 5) and
LCDR3 (SEQ ID NO: 6). In one aspect, the present invention
provides a specific binding member for human IL-13, comprising
an antibody antigen-binding site which is composed of a human

antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the HCDR1 has the amino acid sequence of

5   SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6; or wherein

10 the set of CDR's contains one or two amino acid substitutions compared with the set of CDR's, wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid

15 sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6.

The set of CDR's wherein the HCDR1 has the amino acid sequence

20 of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6, are herein

25 referred to as the "BAK278D6 set of CDR's". The HCDR1, HCDR2 and HCDR3 within the BAK278D6 set of CDR's are referred to as the "BAK278D6 set of HCDR's" and the LCDR1, LCDR2 and LCDR3 within the BAK278D6 set of CDR's are referred to as the "BAK278D6 set of LCDR's". A set of CDR's with the BAK278D6

30 set of CDR's, BAK278D6 set of HCDR's or BAK278D6 LCDR's, or one or two substitutions therein, is said to be of the BAK278D6 lineage.

As noted, in one aspect the invention provides a specific binding member for human IL-13, comprising an antibody antigen-binding site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, wherein the set of CDR's is the BAK278D6 set of CDR's or a set of CDR's containing one or two substitutions compared with the BAK278D6 set of CDR's.

In preferred embodiments, the one or two substitutions are at one or two of the following residues within the CDRs of the VH and/or VL domains, using the standard numbering of Kabat [107].

31, 32, 34 in HCDR1

52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2

96, 97, 98, 99, 101 in HCDR3

26, 27, 28, 30, 31 in LCDR1

56 in LCDR2

95A, 97 in LCDR3

Preferred embodiments have two substitutions compared with the BAK278D6 set of CDR's, at HCDR3 residue 99 and LCDR1 residue 27. Of these embodiments, preferred embodiments have S substituted for N at HCDR3 residue 99 and/or I substituted for N at LCDR 1 residue 27. Still further embodiments have a substitution at HCDR3 residue 99 selected from the group consisting of S, A, I, R, P and K, and/or a substitution at LCDR1 residue 27 selected from the group consisting of I, L, M, C, V, K, Y, F, R, T, S, A, H and G.

In preferred embodiments one or two substitutions are made at one or two of the following residues within the BAK278D6 set of CDR's in accordance with the identified groups of possible
5 substitute residues:

Position of substitution	Substitute Residue selected from the group consisting of

10

31 in HCDR1:	Q, D, L, G and E
32 in HCDR1:	T
34 in HCDR1:	V, I and F

15

52 in HCDR2:	D, N, A, R, G and E
52A in HCDR2:	D, G, T, P, N and Y
53 in HCDR2:	D, L, A, P, T, S, I and R
54 in HCDR2:	S, T, D, G, K and I
20 56 in HCDR2:	T, E, Q, L, Y, N, V, A, M and G
58 in HCDR2:	I, L, Q, S, M, H, D and K
60 in HCDR2:	R
61 in HCDR2:	R
25 62 in HCDR2:	K and G
64 in HCDR2:	R
65 in HCDR2:	K
96 in HCDR3:	R and D
30 97 in HCDR3:	N, D, T and P
98 in HCDR3:	R
99 in HCDR3:	S,A, I, R, P and K
101 in HCDR3:	Y

```
   26 in LCDR1:    D and S
   27 in LCDR1:    I, L, M, C, V, K, Y, F, R, T, S, A, H and G
   28 in LCDR1:    V
   30 in LCDR1:    G
5  31 in LCDR1:    R


   56 in LCDR2:    T


   95A in LCDR3:  N
10 97 in LCDR3:   I
```

Preferred embodiments have the BAK278D6 set of CDR's with a substitution of S for N at residue 99 within HCDR3 and I for N at residue 27 within LCDR 1. The set of CDR's thus defined is

15 as follows: HCDR1 - SEQ ID NO: 7; HCDR2 - SEQ ID NO: 8, HCDR3 - SEQ ID NO: 9; LCDR1 - SEQ ID NO: 10, LCDR2 - SEQ ID NO: 11; LCDR3 - SEQ ID NO: 12. This set of CDR's is herein referred to as the "BAK502G9 set of CDR's".

20 Further preferred embodiments have the BAK278D6 set of CDR's with one or two substitutions within the CDR's, with the proviso that the pair of substitutions of S for N at residue 99 within HCDR3 and I for N at residue 27 within LCDR 1 is excluded.

25

Other preferred embodiments are as follows: BAK 1166G2: HCDR1- SEQ ID NO: 67, HCDR2- SEQ ID NO: 68, HCDR3- SEQ ID NO: 69, LCDR1 - SEQ ID NO: 70, LCDR2 - SEQ ID NO: 71; LCDR3 - SEQ ID NO: 72.

30

BAK1167F2 HCDR1- SEQ ID NO: 61, HCDR2- SEQ ID NO:62, HCDR3- SEQ ID NO:63, LCDR1 - SEQ ID NO: 64, LCDR2 - SEQ ID NO: 65; LCDR3 - SEQ ID NO: 66.

BAK1184C8: HCDR1- SEQ ID NO:73, HCDR2: SEQ ID NO:74, HCDR3- SEQ ID NO:75. LCDR1 - SEQ ID NO: 76, LCDR2 - SEQ ID NO: 77; LCDR3 - SEQ ID NO: 78.

5 BAK1185E1: HCDR1- SEQ ID NO:79, HCDR2- SEQ ID NO:80, HCDR3- SEQ ID NO: 81. LCDR1 - SEQ ID NO: 82, LCDR2 - SEQ ID NO: 83; LCDR3 - SEQ ID NO: 84.

BAK1167F4: HCDR1- SEQ ID NO: 85, HCDR2- SEQ ID NO:86, HCDR3- 10 SEQ ID NO:87. LCDR1 - SEQ ID NO: 88, LCDR2 - SEQ ID NO: 89; LCDR3 - SEQ ID NO: 90.

BAK1111D10: HCDR1- SEQ ID NO: 91, HCDR2- SEQ ID NO: 92, HCDR3- SEQ ID NO: 93. LCDR1 - SEQ ID NO: 94, LCDR2 - SEQ ID NO: 95; 15 LCDR3 - SEQ ID NO: 96.

BAK1183H4: HCDR1- SEQ ID NO: 97, HCDR2- SEQ ID NO: 98, HCDR3- SEQ ID NO: 99. LCDR1 - SEQ ID NO: 100, LCDR2 - SEQ ID NO: 101; LCDR3 - SEQ ID NO: 102.

20

BAK1185F8: HCDR1- SEQ ID NO: 103, HCDR2- SEQ ID NO: 104, HCDR3- SEQ ID NO: 105. LCDR1 - SEQ ID NO: 106, LCDR2 - SEQ ID NO: 107; LCDR3 - SEQ ID NO: 108. All of these were derived from 502G9 by heavy chain CDR1 and CDR2 randomisation and are 25 thus of the BAK502G9 lineage.

A VH domain comprising a set of CDR's HCDR1, HCDR2 and HCDR3 of any clone as shown in Table 1. Table 1 is also provided by the present invention, as is separately a VL domain comprising 30 a set of CDR's LCDR1, LCDR2 and LCDR3 of the clones shown in Table 1. Preferably such a VH domain is paired with such a VL domain, and most preferably the VH and VL domain pairings are the same as in the clones as set out in Table 1.

Further provided by the present invention is a VH domain
comprising a set of CDR's HCDR1, HCDR2 and HCDR3 wherein the
set of CDR's corresponds to that for any clone shown in Table
1 with one or two amino acid substitutions.

5

Further provided by the present invention is a VL domain
comprising a set of CDR's LCDR1, LCDR2 and LCDR3 wherein the
set of CDR's corresponds to that for any clone shown in Table
1 with one or two amino acid substitutions.

10

A specific binding member comprising an antibody antigen-
binding domain comprising such a VH and/or VL domain is also
provided by the present invention.

15 The present inventors have identified the BAK278D6 lineage as
providing human antibody antigen-binding domains against IL-13
which are of particular value. Within the lineage, BAK502G9
has been identified to be of special value. The BAK278D6 and
BAK502G9 sets of CDR's have been identified already above.

20

Following the lead of computational chemistry in applying
multivariate data analysis techniques to the
structure/property-activity relationships [94], quantitative
activity-property relationships of antibodies can be derived
25 using well-known mathematical techniques such as statistical
regression, pattern recognition and classification [95-100].
The properties of antibodies can be derived from empirical and
theoretical models (for example, analysis of likely contact
residues or calculated physicochemical property) of antibody
30 sequence, functional and three-dimensional structures and
these properties can be considered singly and in combination.

An antibody antigen-binding site composed of a VH domain and a
VL domain is formed by six loops of polypeptide: three from

the light chain variable domain (VL) and three from the heavy
chain variable domain (VH). Analysis of antibodies of known
atomic structure has elucidated relationships between the
sequence and three-dimensional structure of antibody combining
5   sites[101,102]. These relationships imply that, except for the
third region (loop) in VH domains, binding site loops have one
of a small number of main-chain conformations: canonical
structures. The canonical structure formed in a particular
loop has been shown to be determined by its size and the
10   presence of certain residues at key sites in both the loop and
in framework regions [101,102].

This study of sequence-structure relationship can be used for
prediction of those residues in an antibody of known sequence,
15   but of an unknown three-dimensional structure, which are
important in maintaining the three-dimensional structure of
its CDR loops and hence maintain binding specificity. These
predictions can be backed up by comparison of the predictions
to the output from lead optimization experiments.
20

In a structural approach, a model can be created of the
antibody molecule [103] using an freely available or
commercial package such as WAM [104]. A protein visualisation
and analysis software package such as Insight II [105] or Deep
25   View [106] may then be used to evaluate possible substitutions
at each position in the CDR. This information may then be used
to make substitutions likely to have a minimal or beneficial ·
effect on activity.

30   The present inventors analysed sequence data of the panel of
clones for which the sets of CDR's are shown in Table 1.

The analysis tested the hypothesis that any binary
combinations of listed amino acid variations in the CDR's from

the presented set of scFv variants leads to a scFv variant with at least the starting potency of the parent scFv BAK278D6.

5   All scFv variants in the panel shown in Table 1 have been selected for improved affinity and have been confirmed to display higher potency.

The observed amino acid variations can either be favourable,
10   non-favourable or neutral in their effect on the starting potency of scFv BAK278D6 in the TF-1 assay of 44nM.

No linkage was observed between any two amino acid variations confirming that there was no synergy, either "positive" or
15   "negative", between any two selected amino acid variations.

There are four scenarios where such binary combination will fulfil the hypothesis and three scenarios where the hypothesis will not be valid. Synergistic amino acid variants are not
20   considered as no linkage was observed.

The hypothesis is valid where:

A1: mutation 1 is favourable and mutation 2 is favourable
25   A2 : mutation 1 is favourable and mutation 2 is neutral
A3: mutation 1 is neutral and mutation 2 is neutral
A4: mutation 1 is favourable and mutation 2 is non-favourable
(with the effect of 1 outweighing the effect of 2) .

30   The hypothesis is not valid where:

B1: mutation 1 is non-favourable and mutation 2 is neutral
B2: mutation 1 is non-favourable and mutation 2 is non-favourable

B3: mutation 1 is favourable and mutation 2 is non-favourable (with the effect of 2 outweighing the effect of 1).

For A4 to be possible, mutation 1 needs to be highly
5 favourable to counterbalance the negative effect of mutation 2 on potency. Since such highly favourable mutation would be present in the library of variants used for selection, it would be selected for and would therefore appear frequently in the panel of variants. Since synergy can be excluded, such
10 mutation would be beneficial in any kind of sequence context and should therefore reappear in different scFv variants. An example for such frequent amino acid change is the change in the light chain CDR1 Asn27Ile. However, this mutation on its own (in clone BAK531E2) has only a modest 2-fold effect on
15 potency (final IC50 of 23.2nM). On its own this mutation would not allow the scenario depicted in A4, as it is not a highly favourable mutation. This suggests that every clone in the presented set of IL-13 binding clones (Table 1) which has a light chain CDR1 Asn27Ile change along with one or more
20 further mutations is at least as potent as the variant having the single light chain CDR1 Asn27Ile mutation. The other mutations are either neutral or positive but do not have a negative or detrimental affect.

25 A further example is in the heavy chain CDR3 Asn99Ser (see Table 1). As a clone carrying this particular single amino acid variation is not observed, the potency of such a clone has been estimated to be approximately 12.0nM by the following rationale:
30

BAK278D6 potency is 44nM. Alterations of VL CDR1 N27I + VH CDR3 N99S lead to BAK502G9 with potency 8nM, i.e. 5.5 fold improvement.

BAK278D6 potency is 44nM. Alteration of VL CDR1 N27I leads to
BAK531E2 with potency 23 nM, i.e. 1.9 fold improvement

BAK278D6 potency is 44nM. Alteration VH CDR3 N99S to provide
5   a possible clone with potency 12.2nM, i.e. 2.9 fold
improvement (5.5/1.9 = 2.9).

The binary combination of heavy chain CDR3 Asn99Ser with light
chain CDR1 Asn27Ile gives a scFv BAK0502G9 with a potency of
10 8nM. As synergy is excluded, the contribution of heavy chain
CDR3 Asn99Ser change in BAK502G9 is therefore additive.

Therefore every clone in the presented set of IL-13 binding
clones (Table 1) which has a heavy chain CDR3 AsnH99Ser change
15 along with one or more further mutations would have a potency
of at least 12nM or greater.

Thus, the inventors note that a highly favourable amino acid
variation which would be selected preferentially is not
20 observed. As discussed above, two variations which were
prominently represented in Table 1 of scFv variants were
analysed closer. Any scFv variant in Table 1 with either of
these mutations along with one or more further mutations
displayed a potency which was at least as improved as a clone
25 containing any one of these two single amino acid variations
in the parent BAK278D6. There is therefore no evidence that a
highly favourable amino acid variation, that would allow
scenario A4, is present in the panel.

30 This observation led the inventors to conclude that there were
no non-favourable mutations present in this set of scFv
variants. This means scenarios A4 and B1 to B3 are not
relevant and the hypothesis is valid.

Accordingly, as noted already, the present invention provides specific binding members comprising the defined sets of CDR's, in particular the set of CDR's of BAK278D6, and sets of CDR's of the BAK278D6 lineage, with one or two substitutions within
5   the set of CDR's, e.g. the BAK502G9 set of CDR's.

The relevant set of CDR's is provided within antibody framework regions or other protein scaffold, e.g. fibronectin or cytochrome B [115, 116]. Preferably antibody framework
10   regions are employed, and where they are employed they are preferably germline, more preferably the antibody framework region for the heavy chain may be DP14 from the VH1 family. The preferred framework region for the light chain may be λ3-3H.   For the BAK502G9 set of CDR's it is preferred that the
15   antibody framework regions are for VH FR1, SEQ ID NO: 27, for VH FR2, SEQ ID NO: 28, for VH FR3, SEQ ID NO 29, for light chain FR1, SEQ ID NO: 30, for light chain FR2, SEQ ID NO: 31, for light chain FR3, SEQ ID NO: 32. In a highly preferred embodiment, a VH domain is provided with the amino acid
20   sequence of SEQ ID NO: 15, this being termed "BAK502G9 VH domain". In a further highly preferred embodiment, a VL domain is provided with the amino acid sequence of SEQ ID NO: 16, this being termed "BAK502G9 VL domain". A highly preferred antibody antigen-binding site provided in accordance
25   with the present invention is composed of the BAK502G9 VH domain, SEQ ID NO: 15, and the BAK502G9 VL domain, SEQ ID NO: 16. This antibody antigen-binding site may be provided within any desired antibody molecule format, e.g. scFv, Fab, IgG, IgG4, dAb etc., as is discussed further elsewhere herein.
30

In a further highly preferred embodiment, the present invention provides an IgG4 antibody molecule comprising the BAK502G9 VH domain, SEQ ID NO: 15, and the BAK502G9 VL domain, SEQ ID NO: 16. This is termed herein "BAK502G9 IgG4".

Other IgG4 or other antibody molecules comprising the BAK502G9 VH domain, SEQ ID NO: 15, and/or the BAK502G9 VL domain, SEQ ID NO: 16, are provided by the present invention, as are other

5 antibody molecules comprising the BAK502G9 set of HCDR's (SEQ ID NO: 7, 8 and 9) within an antibody VH domain, and/or the BAK502G9 set of LCDR's (SEQ ID NO: 10, 11 and 12) within an antibody VL domain.

10 It is convenient to point out here that "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as

15 if each is set out individually herein.

As noted, the present invention provides a specific binding member which binds human IL-13 and which comprises the BAK502G9 VH domain (SEQ ID NO: 15) and/or the BAK502G9 VL

20 domain (SEQ ID NO: 16).

Generally, a VH domain is paired with a VL domain to provide an antibody antigen binding site, although as discussed further below a VH domain alone may be used to bind antigen.

25 In one preferred embodiment, the BAK502G9 VH domain (SEQ ID NO: 15) is paired with the BAK502G9 VL domain (SEQ ID NO: 16), so that an antibody antigen binding site is formed comprising both the BAK502G9 VH and VL domains. In other embodiments, the BAK502G9 VH is paired with a VL domain other than the

30 BAK502G9 VL. Light-chain promiscuity is well established in the art.

Similarly, any set of HCDR's of the BAK278D6 lineage can be provided in a VH domain that is used as a specific binding

member alone or in combination with a VL domain. A VH domain
may be provided with a set of HCDR's of a BAK278D6 lineage
antibody, e.g. as shown in Table 1, and if such a VH domain is
paired with a VL domain, then the VL domain may be provided
5   with a set of LCDR's of a BAK278D6 lineage antibody, e.g. as
shown in Table 1. A pairing of a set of HCDR's and a set of
LCDR's may be as shown in Table 1, providing an antibody
antigen-binding site comprising a set of CDR's as shown in
Table 1. The framework regions of the VH and/or VL domains
10   may be germline frameworks. Frameworks regions of the heavy
chain domain may be selected from the VH-1 family, and a
preferred VH-1 framework is DP-14 framework. Framework regions
of the light chain may be selected from the λ3 family, and a
preferred such framework is λ3 3H.
15

One or more CDRs may be taken from the BAK502G9 VH or VL
domain and incorporated into a suitable framework. This is
discussed further herein. BAK502G9 HCDR's 1, 2 and 3 are
shown in SEQ ID NO: 7, 8 and 9, respectively. BAK502G9 LCDR's
20   1, 2 and 3 are shown in SEQ ID NO: 10, 11 and 12,
respectively.

The same applies for other BAK278D6 lineage CDR's and sets of
CDR's as shown in Table 1.
25

Further embodiments of the invention relate to a specific
binding member comprising the VH and/or VL domain, or an
antigen binding site comprising CDRs of the VH and/or VL
domain of the antibody molecule disclosed herein as
30   167A11 (VH: SEQ ID NO: 23 and VL: SEQ ID NO: 24) and its
derivatives 615E3 (VH:SEQ ID NO: 33 and VL: SEQ ID NO: 34)
BAK582F7 (VH CDR's SEQ ID's 141-143) and BAK612B5 (VH CDR's
SEQ ID's 147-149). These recognise human IL-13. The
derivatives of 167A11 from VH CDR3 randomisation are potent

scFv molecules (5-6nM).. The 167A11 lineage may be employed in
any aspect and embodiment of the present invention as
disclosed herein for other molecules, for instance methods of
mutation and selection of antigen binding sites with improved
5   potency.

Variants of the VH and VL domains and CDRs of the present
invention, including those for which amino acid sequences are
set out herein, and which can be employed in specific binding
10   members for IL-13 can be obtained by means of methods of
sequence alteration or mutation and screening. Such methods
are also provided by the present invention.

Variable domain amino acid sequence variants of any of the VH
15   and VL domains whose sequences are specifically disclosed
herein may be employed in accordance with the present
invention, as discussed. Particular variants may include one
or more amino acid sequence alterations (addition, deletion,
substitution and/or insertion of an amino acid residue), may
20   be less than about 20 alterations, less than about 15
alterations, less than about 10 alterations or less than about
5 alterations, 4, 3, 2 or 1. Alterations may be made in one
or more framework regions and/or one or more CDR's.

25   In accordance with further aspects of the present invention
there is provided a specific binding member which competes for
binding to antigen with any specific binding member which both
binds the antigen and comprises a specific binding member, VH
and/or VL domain disclosed herein, or HCDR3 disclosed herein,
30   or variant of any of these. Competition between binding
members may be assayed easily in vitro, for example using
ELISA and/or by tagging a specific reporter molecule to one
binding member which can be detected in the presence of other
untagged binding member(s), to enable identification of

specific binding members which bind the same epitope or an
overlapping epitope.

Thus, a further aspect of the present invention provides a
5 specific binding member comprising a human antibody antigen-
binding site which competes with a BAK502G9 antibody molecule,
in particular BAK502G9 scFv and/or IgG4, for binding to IL-13.
In further aspects the present invention provides a specific
binding member comprising a human antibody antigen-binding
10 site which competes with an antibody antigen-binding site for
binding to IL-13, wherein the antibody antigen-binding site is
composed of a VH domain and a VL domain, and wherein the VH
and VL domains comprise a set of CDR's of the BAK278D6
lineage.
15

Various methods are available in the art for obtaining
antibodies against IL-13 and which may compete with a BAK502G9
antibody molecule, an antibody molecule with a BAK502G9 set of
CDR's, or an antibody molecule with a set of CDR's of BAK278D6
20 lineage, for binding to IL-13.

In a further aspect, the present invention provides a method
of obtaining one or more specific binding members able to bind
the antigen, the method including bringing into contact a
25 library of specific binding members according to the invention
and said antigen, and selecting one or more specific binding
members of the library able to bind said antigen.

The library may be displayed on the surface of bacteriophage
30 particles, each particle containing nucleic acid encoding the
antibody VH variable domain displayed on its surface, and
optionally also a displayed VL domain if present.

Following selection of specific binding members able to bind
the antigen and displayed on bacteriophage particles, nucleic
acid may be taken from a bacteriophage particle displaying a
said selected specific binding member. Such nucleic acid may
be used in subsequent production of a specific binding member
or an antibody VH variable domain (optionally an antibody VL
variable domain) by expression from nucleic acid with the
sequence of nucleic acid taken from a bacteriophage particle
displaying a said selected specific binding member.

An antibody VH variable domain with the amino acid sequence of
an antibody VH variable domain of a said selected specific
binding member may be provided in isolated form, as may a
specific binding member comprising such a VH domain.
Ability to bind IL-13 may be further tested, also ability to
compete with BAK502G9 (e.g. in scFv format and/or IgG format,
e.g. IgG4) for binding to IL-13. Ability to neutralise IL-13
may be tested, as discussed further below.

A specific binding member according to the present invention
may bind IL-13 with the affinity of a BAK502G9 antibody
molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with an
affinity that is better.

A specific binding member according to the present invention
may neutralise IL-13 with the potency of a BAK502G9 antibody
molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with a
potency that is better.

A specific binding member according to the present invention
may neutralise naturally occurring IL-13 with the potency of a
BAK502G9 antibody molecule, e.g. scFv, or preferably BAK502G9
IgG4, or with a potency that is better.

Binding affinity and neutralisation potency of different specific binding members can be compared under appropriate conditions.

5   The antibodies of the present invention have a number of advantages over existing commercial anti-IL-13 antibodies, in particular three commercial rodent anti-human IL-13 antibodies namely, JES10-5A2 (BioSource), B-B13 (Euroclone) and clone 321166 (R&D Systems). The potency of the antibodies of the
10   present invention was compared with commercial antibodies JES10-A2 and B-B13. Clone 321166 was not evaluated as previous experiments revealed that this clone was considerably less potent than other known commercial antibodies.

15   The efficacy and use of the rodent commercial IL-13 antibodies in man is likely to be limited, because of their increased potential to induce immunogenic responses and therefore more rapid clearance from the body. Kinetic analysis of the antibodies of the present invention in non-human primates
20   suggests that these antibodies have a clearance rate which is similar to that of other known human or humanised antibodies.

Antibodies provided by various embodiments of the present invention recognize non-human primate IL-13, including rhesus
25   and cynomolgus IL-13. Determining efficacy and safety profiles of an antibody in non-human primates is extremely valuable as it provides a means for predicting the antibody's safety, pharmacokinetic and pharmacodynamic profile in humans.

30   Moreover, antibodies of various embodiments of the present invention further recognize the human IL-13 variant, Q130R, which is associated with asthma. Cross reactivity with variant IL-13 allows antibodies of the present invention and compositions comprising antibodies of the present invention to

be used for the treatment of patients with wild-type and variant IL-13.

A preferred embodiment of the present invention comprises antibodies that neutralise naturally occurring IL-13 with a potency that is equal to or better than the potency of a IL-13 antigen binding site formed by BAK502G9 VH domain (SEQ ID NO:15) and the BAK502G9 VL domain (SEQ ID NO: 16). For example, the inventors have demonstrated that representative clones such as BAK502G9, 1167F2 and 1183H4 are significantly more potent against naturally occurring IL-13 than known commercial antibodies (Figure 7).

In addition to antibody sequences, a specific binding member according to the present invention may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Specific binding members of the invention may carry a detectable label, or may be conjugated to a toxin or a targeting moiety or enzyme (e.g. via a peptidyl bond or linker).

In further aspects, the invention provides an isolated nucleic acid which comprises a sequence encoding a specific binding member, VH domain and/or VL domains according to the present invention, and methods of preparing a specific binding member, a VH domain and/or a VL domain of the invention, which comprise expressing said nucleic acid under conditions to bring about production of said specific binding member, VH domain and/or VL domain, and recovering it.

Specific binding members according to the invention may be used in a method of treatment or diagnosis of the human or

animal body, such as a method of treatment (which may include prophylactic treatment) of a disease or disorder in a human patient which comprises administering to said patient an effective amount of a specific binding member of the

5   invention. Conditions treatable in accordance with the present invention include any in which IL-13 plays a role, especially asthma, atopic dermatitis, allergic rhinitis, fibrosis, chronic obstructive pulmonary disease, scleroderma, inflammatory bowel disease and Hodgkin's lymphoma. Further,

10 the antibodies of the present invention may also be used in treating tumours and viral infections as these antibodies will inhibit IL-13 mediated immunosupression [64, 65].

A further aspect of the present invention provides nucleic
15 acid, generally isolated, encoding an antibody VH variable domain and/or VL variable domain disclosed herein.

Another aspect of the present invention provides nucleic acid, generally isolated, encoding a VH CDR or VL CDR sequence
20 disclosed herein, especially a VH CDR selected from SEQ ID NO's: 7, 8 and 9 or a VL CDR selected from SEQ ID NO's: 10, 11 and 12, most preferably BAK502G9 VH CDR3 (SEQ ID NO: 9). Nucleic acid encoding the BAK502G9 set of CDR's, nucleic acid encoding the BAK502G9 set of HCDR's and nucleic acid encoding
25 the BAK502G9 set of LCDR's are also provided by the present invention, as are nucleic acids encoding individual CDR's, HCDR's, LCDR's and sets of CDR's, HCDR's, LCDR's of the BAK278D6 lineage.

30 A further aspect provides a host cell transformed with nucleic acid of the invention.

A yet further aspect provides a method of production of an antibody VH variable domain, the method including causing

expression from encoding nucleic acid. Such a method may comprise culturing host cells under conditions for production of said antibody VH variable domain.

5   Analogous methods for production of VL variable domains and specific binding members comprising a VH and/or VL domain are provided as further aspects of the present invention.

A method of production may comprise a step of isolation and/or
10   purification of the product.

A method of production may comprise formulating the product into a composition including at least one additional component, such as a pharmaceutically acceptable excipient.
15

These and other aspects of the invention are described in further detail below.

TERMINOLOGY
20

Specific binding member
This describes a member of a pair of molecules which have binding specificity for one another. The members of a specific binding pair may be naturally derived or wholly or
25   partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have
30   the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate. The present invention is concerned with antigen-antibody type reactions.

Antibody molecule

This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any
5 polypeptide or protein comprising an antibody binding domain. Antibody fragments which comprise an antigen binding domain are molecules such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use
10 techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an
15 antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400, and a large body of subsequent literature. A hybridoma or other cell producing an antibody may be subject to genetic mutation or
20 other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody molecule" should be construed as covering any
25 specific binding member or substance having an antibody antigen-binding domain with the required specificity. Thus, this term covers antibody fragments and derivatives, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric
30 molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023, and a large body of subsequent literature.

Further techniques available in the art of antibody
engineering have made it possible to isolate human and
humanised antibodies. For example, human hybridomas can be
5   made as described by Kontermann et al [107]. Phage display,
another established technique for generating specific binding
members has been described in detail in many publications such
as Kontermann et al [107] and WO92/01047 (discussed further
below). Transgenic mice in which the mouse antibody genes are
10   inactivated and functionally replaced with human antibody
genes while leaving intact other components of the mouse
immune system, can be used for isolating human antibodies to
human antigens [108].

15   Synthetic antibody molecules may be created by expression from
genes generated by means of oligonucleotides synthesized and
assembled within suitable expression vectors, for example as
described by Knappik et al. J. Mol. Biol. (2000) 296, 57-86 or
Krebs et al. Journal of Immunological Methods 254 2001 67-84.
20

It has been shown that fragments of a whole antibody can
perform the function of binding antigens. Examples of binding
fragments are (i) the Fab fragment consisting of VL, VH, CL
and CH1 domains; (ii) the Fd fragment consisting of the VH and
25   CH1 domains; (iii) the Fv fragment consisting of the VL and VH
domains of a single antibody; (iv) the dAb fragment (Ward,
E.S. et al., Nature 341, 544-546 (1989), McCafferty et al
(1990) Nature, 348, 552-554) which consists of a VH domain;
(v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent
30   fragment comprising two linked Fab fragments (vii) single
chain Fv molecules (scFv), wherein a VH domain and a VL domain
are linked by a peptide linker which allows the two domains to
associate to form an antigen binding site (Bird et al,
Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-

5883, 1988); (viii) bispecific single chain Fv dimers
(PCT/US92/09965) and (ix) "diabodies", multivalent or
multispecific fragments constructed by gene fusion
(WO94/13804; P. Holliger et al, Proc. Natl. Acad. Sci. USA 90
5   6444-6448, 1993). Fv, scFv or diabody molecules may be
stabilised by the incorporation of disulphide bridges linking
the VH and VL domains (Y. Reiter et al, Nature Biotech, 14,
1239-1245, 1996). Minibodies comprising a scFv joined to a
CH3 domain may also be made (S. Hu et al, Cancer Res., 56,
10   3055-3061, 1996).

Where bispecific antibodies are to be used, these may be
conventional bispecific antibodies, which can be manufactured
in a variety of ways (Holliger, P. and Winter G. Current
15   Opinion Biotechnol. 4, 446-449 (1993)), e.g. prepared
chemically or from hybrid hybridomas, or may be any of the
bispecific antibody fragments mentioned above. Examples of
bispecific antibodies include those of the BiTE™ technology in
which the binding domains of two antibodies with different
20   specificity can be used and directly linked via short flexible
peptides. This combines two antibodies on a short single
polypeptide chain. Diabodies and scFv can be constructed
without an Fc region, using only variable domains, potentially
reducing the effects of anti-idiotypic reaction.
25

Bispecific diabodies, as opposed to bispecific whole
antibodies, may also be particularly useful because they can
be readily constructed and expressed in E.coli. Diabodies
(and many other polypeptides such as antibody fragments) of
30   appropriate binding specificities can be readily selected
using phage display (WO94/13804) from libraries. If one arm
of the diabody is to be kept constant, for instance, with a
specificity directed against IL-13, then a library can be made
where the other arm is varied and an antibody of appropriate

specificity selected. Bispecific whole antibodies may be made
by knobs-into-holes engineering (J. B. B. Ridgeway et al,
Protein Eng., 9, 616-621, 1996).

5 *Antigen-binding domain*
This describes the part of an antibody molecule which
comprises the area which specifically binds to and is
complementary to part or all of an antigen. Where an antigen
is large, an antibody may only bind to a particular part of
10 the antigen, which part is termed an epitope. An antigen
binding domain may be provided by one or more antibody
variable domains (e.g. a so-called Fd antibody fragment
consisting of a VH domain). Preferably, an antigen binding
domain comprises an antibody light chain variable region (VL)
15 and an antibody heavy chain variable region (VH).

Specific
This may be used to refer to the situation in which one member
of a specific binding pair will not show any significant
20 binding to molecules other than its specific binding
partner(s). The term is also applicable where e.g. an antigen
binding domain is specific for a particular epitope which is
carried by a number of antigens, in which case the specific
binding member carrying the antigen binding domain will be
25 able to bind to the various antigens carrying the epitope.

Comprise
This is generally used in the sense of include, that is to say
permitting the presence of one or more features or components.
30

Isolated
This refers to the state in which specific binding members of
the invention, or nucleic acid encoding such binding members,
will generally be in accordance with the present invention.

Isolated members and isolated nucleic acid will be free or substantially free of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the

5   environment in which they are prepared (e.g. cell culture) when such preparation is by recombinant DNA technology practised *in vitro* or *in vivo*. Members and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the members will

10 normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Specific binding members may be glycosylated, either naturally or by systems of

15 heterologous eukaryotic cells (e.g. CHO or NS0 (ECACC 85110503) cells, or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

Naturally occurring IL-13

20 This generally refers to a state in which the IL-13 protein or fragments thereof may occur. Naturally occurring IL-13 means IL-13 protein which is naturally produced by a cell, without prior introduction of encoding nucleic acid using recombinant technology. Thus, naturally occurring IL-13 may be as

25 produced naturally by for example CD4+ T cells and/or as isolated from a mammal, e.g. human, non-human primate, rodent such as rat or mouse.

Recombinant IL-13

30 This refers to a state in which the IL-13 protein or fragments thereof may occur. Recombinant IL-13 means IL-13 protein or fragments thereof produced by recombinant DNA in a heterologous host. Recombinant IL-13 may differ from naturally occurring IL-13 by glycosylation.

Recombinant proteins expressed in prokaryotic bacterial expression systems are not glycosylated while those expressed in eukaryotic systems such as mammalian or insect cells are glycosylated. Proteins expressed in insect cells however differ in glycosylation from proteins expressed in mammalian cells.

By "substantially as set out" it is meant that the relevant CDR or VH or VL domain of the invention will be either identical or highly similar to the specified regions of which the sequence is set out herein. By "highly similar" it is contemplated that from 1 to 5, preferably from 1 to 4 such as 1 to 3 or 1 or 2, or 3 or 4, amino acid substitutions may be made in the CDR and/or VH or VL domain.

The structure for carrying a CDR or a set of CDR's of the invention will generally be of an antibody heavy or light chain sequence or substantial portion thereof in which the CDR or set of CDR's is located at a location corresponding to the CDR or set of CDR's of naturally occurring VH and VL antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of immunoglobulin variable domains may be determined by reference to (Kabat, E.A. et al, Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (http://immuno.bme.nwu.edu or find "Kabat" using any search engine).

CDR's can also be carried by other scaffolds such as fibronectin or cytochrome B [115, 116].

Preferably, a CDR amino acid sequence substantially as set out herein is carried as a CDR in a human variable domain or a substantial portion thereof. The HCDR3 sequences substantially as set out herein represent preferred

5   embodiments of the present invention and it is preferred that each of these is carried as a HCDR3 in a human heavy chain variable domain or a substantial portion thereof.

Variable domains employed in the invention may be obtained

10   from any germ-line or rearranged human variable domain, or may be a synthetic variable domain based on consensus sequences of known human variable domains. A CDR sequence of the invention (e.g. CDR3) may be introduced into a repertoire of variable domains lacking a CDR (e.g. CDR3), using recombinant DNA

15   technology.

For example, Marks et al (Bio/Technology, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to

20   the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks et al further describe how this repertoire may be combined with a CDR3 of a particular

25   antibody. Using analogous techniques, the CDR3-derived sequences of the present invention may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide specific binding members of the

30   invention. The repertoire may then be displayed in a suitable host system such as the phage display system of WO92/01047 or any of a subsequent large body of literature, including Kay, B.K., Winter, J., and McCafferty, J. (1996) Phage Display of Peptides and Proteins: A Laboratory Manual, San Diego:

Academic Press, so that suitable specific binding members may be selected. A repertoire may consist of from anything from 10^4 individual members upwards, for example from 10^6 to 10^8 or 10^{10} members. Other suitable host systems include yeast

5   display, bacterial display, T7 display, ribosome display and so on.

Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (*Nature*, 1994, 370:389-391), who

10   describes the technique in relation to a β-lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions

15   carrying CDR-derived sequences of the invention using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram *et al* (1992, *Proc. Natl. Acad. Sci.*, *USA*, 89:3576-3580), who used error-prone PCR. In

20   preferred embodiments one or two amino acid substitutions are made within a set of HCDR's and/or LCDR's.

Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed

25   by Barbas *et al*, (1994, *Proc. Natl. Acad. Sci.*, *USA*, 91:3809-3813) and Schier *et al* (1996, *J. Mol. Biol.* 263:551-567).

All the above described techniques are known as such in the art and in themselves do not form part of the present

30   invention. The skilled person will be able to use such techniques to provide specific binding members of the invention using routine methodology in the art.

A further aspect of the invention provides a method for obtaining an antibody antigen binding domain specific for IL-13 antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more

5   amino acids in the amino acid sequence of a VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or VH/VL combination or combinations to identify a

10   specific binding member or an antibody antigen binding domain specific for IL-13 antigen and optionally with one or more preferred properties, preferably ability to neutralise IL-13 activity. Said VL domain may have an amino acid sequence which is substantially as set out herein.

15

An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

20   In a preferred embodiment, BAK502G9 VH domain (SEQ ID NO: 15) may be subject to mutation to provide one or more VH domain amino acid sequence variants, and/or BAK502G9 VL (SEQ ID NO: 16).

25   A further aspect of the invention provides a method of preparing a specific binding member specific for IL-13 antigen, which method comprises:
        (a)   providing a starting repertoire of nucleic acids encoding a VH domain which either include a CDR3 to be

30   replaced or lack a CDR3 encoding region;
        (b)   combining said repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out herein for a VH CDR3 such that said donor nucleic acid is inserted into the CDR3 region in the repertoire, so as to

provide a product repertoire of nucleic acids encoding a VH domain;

(c) expressing the nucleic acids of said product repertoire;

5 (d) selecting a specific binding member specific for a IL-13; and

(e) recovering said specific binding member or nucleic acid encoding it.

10 Again, an analogous method may be employed in which a VL CDR3 of the invention is combined with a repertoire of nucleic acids encoding a VL domain which either include a CDR3 to be replaced or lack a CDR3 encoding region.

15 Similarly, one or more, or all three CDRs may be grafted into a repertoire of VH or VL domains which are then screened for a specific binding member or specific binding members specific for IL-13.

20 In a preferred embodiment, one or more of BAK502G9 HCDR1 (SEQ ID NO: 7), HCDR2 (SEQ ID NO: 8) and HCDR3 (SEQ ID NO: 9), or the BAK502G9 set of HCDR's, may be employed, and/or one or more of BAK502G9 LCDR1 (SEQ ID NO: 10), LCDR2 (SEQ ID NO: 11), or the BAK502G9 set of LCDR's.

25

A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the

30 first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with

naturally occurring variable domain regions. For example, construction of specific binding members of the present invention made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers

5   introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains of the invention to further protein sequences including immunoglobulin heavy chains, other variable domains (for example in the production of diabodies)

10   or protein labels as discussed in more detail elsewhere herein.

Although in a preferred aspect of the invention specific binding members comprising a pair of VH and VL domains are

15   preferred, single binding domains based on either VH or VL domain sequences form further aspects of the invention. It is known that single immunoglobulin domains, especially VH domains, are capable of binding target antigens in a specific manner.

20

In the case of either of the single specific binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain specific binding member able to bind IL-13.

25

This may be achieved by phage display screening methods using the so-called hierarchical dual combinatorial approach as disclosed in WO92/01047, in which an individual colony containing either an H or L chain clone is used to infect a

30   complete library of clones encoding the other chain (L or H) and the resulting two-chain specific binding member is selected in accordance with phage display techniques such as those described in that reference. This technique is also disclosed in Marks *et al, ibid.*

Specific binding members of the present invention may further comprise antibody constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to

5   antibody light chain constant domains including human Cκ or Cλ chains, preferably Cλ chains. Similarly, a specific binding member based on a VH domain may be attached at its C-terminal end to all or part (e.g. a CH1 domain) of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA,

10   IgE and IgM and any of the isotype sub-classes, particularly IgG1 and IgG4. IgG4 is preferred. IgG4 is preferred because it does not bind complement and does not create effector functions. Any synthetic or other constant region variant that has these properties and stabilizes variable regions is

15   also preferred for use in embodiments of the present invention.

Specific binding members of the invention may be labelled with a detectable or functional label. Detectable labels include

20   radiolabels such as ^{131}I or ^{99}Tc, which may be attached to antibodies of the invention using conventional chemistry known in the art of antibody imaging. Labels also include enzyme labels such as horseradish peroxidase. Labels further include chemical moieties such as biotin which may be detected via

25   binding to a specific cognate detectable moiety, e.g. labelled avidin.

Specific binding members of the present invention are designed to be used in methods of diagnosis or treatment in human or

30   animal subjects, preferably human.

Accordingly, further aspects of the invention provide methods of treatment comprising administration of a specific binding member as provided, pharmaceutical compositions comprising

such a specific binding member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition comprising formulating the

5   specific binding member with a pharmaceutically acceptable excipient.

Clinical indications in which an anti-IL-13 antibody may be used to provide therapeutic benefit include asthma, atopic

10   dermatitis, allergic rhinitis, fibrosis, chronic obstructive pulmonary disease, inflammatory bowel disease, scleroderma and Hodgkin's lymphoma . As already explained, anti-IL-13 treatment is effective for all these diseases.

15   Anti-IL-13 treatment may be given orally, by injection (for example, subcutaneously, intravenously, intraperitoneal or intramuscularly), by inhalation, or topically (for example intraocular, intranasal, rectal, into wounds, on skin). The route of administration can be determined by the

20   physicochemical characteristics of the treatment, by special considerations for the disease or by the requirement to optimise efficacy or to minimise side-effects.

It is envisaged that anti-IL-13 treatment will not be

25   restricted to use in the clinic. Therefore, subcutaneous injection using a needle free device is also preferred.

Combination treatments may be used to provide significant synergistic effects, particularly the combination of an anti-

30   IL-13 specific binding member with one or more other drugs. A specific binding member according to the present invention may be provided in combination or addition to short or long acting beta agonists, corticosteroids, cromoglycate, leukotriene (receptor) antagonists, methyl xanthines and their

derivatives, IL-4 inhibitors, muscarinic receptor antagonists, IgE inhibitors, histaminic inhibitors, IL-5 inhibitors, eotaxin/CCR3 inhibitors, PDE4 inhibitors, TGF-beta antagonists, interferon-gamma, perfenidone, chemotherapeutic
5 agents and immunotherapeutic agents.

Combination treatment with one or more short or long acting beta agonists, corticosteroids, cromoglycate, leukotriene (receptor) antagonists, xanthines, IgE inhibitors, IL-4
10 inhibitors, IL-5 inhibitors, eotaxin/CCR3 inhibitors, PDE4 inhibitors may be employed for treatment of asthma. Antibodies of the present invention can also be used in combination with corticosteroids, anti-metabolites, antagonists of TGF-beta and its downstream signalling pathway, for treatment of fibrosis.
15 Combination therapy of these antibodies with PDE4 inhibitors, xanthines and their derivatives, muscarinic receptor antagonists, short and long beta antagonists can be useful for treating chronic obstructive pulmonary disease. Similar consideration of combinations apply to the use of anti-IL-13
20 treatment for atopic dermatitis, allergic rhinitis, chronic obstructive pulmonary disease, inflammatory bowel disease, scleroderma and Hodgkin's lymphoma.

In accordance with the present invention, compositions
25 provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of
30 administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors. Appropriate doses of antibody are well known in the art; see Ledermann J.A. et al.

(1991) Int. J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991)
Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-
922.

5   The precise dose will depend upon a number of factors,
including whether the antibody is for diagnosis or for
treatment, the size and location of the area to be treated,
the precise nature of the antibody (e.g. whole antibody,
fragment or diabody), and the nature of any detectable label

10 or other molecule attached to the antibody. A typical
antibody dose will be in the range 100µg to 1 gm for systemic
applications, and 1µg to 1mg for topical applications.
Typically, the antibody will be a whole antibody, preferably
the IgG4 isotype. This is a dose for a single treatment of an

15 adult patient, which may be proportionally adjusted for
children and infants, and also adjusted for other antibody
formats in proportion to molecular weight. Treatments may be
repeated at daily, twice-weekly, weekly or monthly intervals,
at the discretion of the physician. In preferred embodiments

20 of the present invention, treatment is periodic, and the
period between administrations is about two weeks or more,
preferably about three weeks or more, more preferably about
four weeks or more, or about once a month.

25 Specific binding members of the present invention will usually
be administered in the form of a pharmaceutical composition,
which may comprise at least one component in addition to the
specific binding member.

30 Thus pharmaceutical compositions according to the present
invention, and for use in accordance with the present
invention, may comprise, in addition to active ingredient, a
pharmaceutically acceptable excipient, carrier, buffer,
stabiliser or other materials well known to those skilled in

the art. Such materials should be non-toxic and should not
interfere with the efficacy of the active ingredient. The
precise nature of the carrier or other material will depend on
the route of administration, which may be oral, or by
5   injection, e.g. intravenous.

Pharmaceutical compositions for oral administration may be in
tablet, capsule, powder or liquid form. A tablet may comprise
a solid carrier such as gelatin or an adjuvant. Liquid
10 pharmaceutical compositions generally comprise a liquid
carrier such as water, petroleum, animal or vegetable oils,
mineral oil or synthetic oil. Physiological saline solution,
dextrose or other saccharide solution or glycols such as
ethylene glycol, propylene glycol or polyethylene glycol may
15 be included.

For intravenous injection, or injection at the site of
affliction, the active ingredient will be in the form of a
parenterally acceptable aqueous solution which is pyrogen-free
20 and has suitable pH, isotonicity and stability. Those of
relevant skill in the art are well able to prepare suitable
solutions using, for example, isotonic vehicles such as Sodium
Chloride Injection, Ringer's Injection, Lactated Ringer's
Injection. Preservatives, stabilisers, buffers, antioxidants
25 and/or other additives may be included, as required.

A composition may be administered alone or in combination with
other treatments, either simultaneously or sequentially
dependent upon the condition to be treated.
30

Specific binding members of the present invention may be
formulated in liquid or solid forms depending on the
physicochemical properties of the molecule and the route of
delivery. Formulations may include excipients, or combinations

of excipients, for example: sugars, amino acids and surfactants. Liquid formulations may include a wide range of antibody concentrations and pH. Solid formulations may be produced by lyophilisation, spray drying, or drying by

5   supercritical fluid technology, for example. Formulations of anti-IL-13 will depend upon the intended route of delivery: for example, formulations for pulmonary delivery may consist of particles with physical properties that ensure penetration into the deep lung upon inhalation; topical formulations may

10 include viscosity modifying agents, which prolong the time that the drug is resident at the site of action.

The present invention provides a method comprising causing or allowing binding of a specific binding member as provided

15 herein to IL-13. As noted, such binding may take place *in vivo*, e.g. following administration of a specific binding member, or nucleic acid encoding a specific binding member, or it may take place *in vitro*, for example in ELISA, Western blotting, immunocytochemistry, immuno-precipitation, affinity

20 chromatography, or cell based assays such as a TF-1 assay.

The amount of binding of specific binding member to IL-13 may be determined. Quantitation may be related to the amount of the antigen in a test sample, which may be of diagnostic

25 interest.

A kit comprising a specific binding member or antibody molecule according to any aspect or embodiment of the present invention is also provided as an aspect of the present

30 invention. In a kit of the invention, the specific binding member or antibody molecule may be labelled to allow its reactivity in a sample to be determined, e.g. as described further below. Components of a kit are generally sterile and in sealed vials or other containers. Kits may be employed in

diagnostic analysis or other methods for which antibody molecules are useful. A kit may contain instructions for use of the components in a method, e.g. a method in accordance with the present invention. Ancillary materials to assist in

5   or to enable performing such a method may be included within a kit of the invention.

The reactivities of antibodies in a sample may be determined by any appropriate means. Radioimmunoassay (RIA) is one

10   possibility. Radioactive labelled antigen is mixed with unlabelled antigen (the test sample) and allowed to bind to the antibody. Bound antigen is physically separated from unbound antigen and the amount of radioactive antigen bound to the antibody determined. The more antigen there is in the

15   test sample the less radioactive antigen will bind to the antibody. A competitive binding assay may also be used with non-radioactive antigen, using antigen or an analogue linked to a reporter molecule. The reporter molecule may be a fluorochrome, phosphor or laser dye with spectrally isolated

20   absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

25   Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or

30   otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral

absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

5

The signals generated by individual antibody-reporter conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples (normal and test).

10

The present invention also provides the use of a specific binding member as above for measuring antigen levels in a competition assay, that is to say a method of measuring the level of antigen in a sample by employing a specific binding

15 member as provided by the present invention in a competition assay. This may be where the physical separation of bound from unbound antigen is not required. Linking a reporter molecule to the specific binding member so that a physical or optical change occurs on binding is one possibility. The

20 reporter molecule may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant

25 expression of a gene fusion encoding antibody and reporter molecule.

The present invention also provides for measuring levels of antigen directly, by employing a specific binding member

30 according to the invention for example in a biosensor system.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to

choose a suitable mode according to their preference and general knowledge.

As noted, in various aspects and embodiments, the present
5   invention extends to a specific binding member which competes for binding to IL-13 with any specific binding member defined herein, e.g. BAK502G9 IgG4. Competition between binding members may be assayed easily *in vitro*, for example by tagging a specific reporter molecule to one binding member which can
10   be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

Competition may be determined for example using ELISA in which
15   IL-13 is immobilised to a plate and a first tagged binding member along with one or more other untagged binding members is added to the plate. Presence of an untagged binding member that competes with the tagged binding member is observed by a decrease in the signal emitted by the tagged binding member.
20

In testing for competition a peptide fragment of the antigen may be employed, especially a peptide including an epitope of interest. A peptide having the epitope sequence plus one or more amino acids at either end may be used. Such a peptide
25   may be said to "consist essentially" of the specified sequence. Specific binding members according to the present invention may be such that their binding for antigen is inhibited by a peptide with or including the sequence given. In testing for this, a peptide with either sequence plus one
30   or more amino acids may be used.

Specific binding members which bind a specific peptide may be isolated for example from a phage display library by panning with the peptide(s).

5   The present invention further provides an isolated nucleic acid encoding a specific binding member of the present invention. Nucleic acid may include DNA and/or RNA. In a preferred aspect, the present invention provides a nucleic acid which codes for a CDR or set of CDR's or VH domain or VL

10   domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG4, of the invention as defined above.

The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which

15   comprise at least one polynucleotide as above.

The present invention also provides a recombinant host cell which comprises one or more constructs as above. A nucleic acid encoding any CDR or set of CDR's or VH domain or VL

20   domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG4 as provided, itself forms an aspect of the present invention, as does a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently

25   be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression a VH or VL domain, or specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

30

Specific binding members, VH and/or VL domains, and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or

homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may

5 comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires

10 otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, plant cells, yeast

15 and baculovirus systems and transgenic plants and animals. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells, YB2/0 rat myeloma cells, human embryonic

20 kidney cells, human embryonic retina cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the

25 art. For a review, see for example Plückthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a specific binding memberfor example Chadd HE and Chamow SM (2001) 110 Current Opinion in

30 Biotechnology 12: 188-194, Andersen DC and Krummen L (2002) Current Opinion in Biotechnology 13: 117, Larrick JW and Thomas DW (2001) Current opinion in Biotechnology 12:411-418.

Suitable vectors can be chosen or constructed, containing
appropriate regulatory sequences, including promoter
sequences, terminator sequences, polyadenylation sequences,
enhancer sequences, marker genes and other sequences as
5   appropriate. Vectors may be plasmids, viral e.g. 'phage, or
phagemid, as appropriate. For further details see, for
example, *Molecular Cloning: a Laboratory Manual*: 3rd edition,
Sambrook and Russell, 2001, Cold Spring Harbor Laboratory
Press. Many known techniques and protocols for manipulation
10   of nucleic acid, for example in preparation of nucleic acid
constructs, mutagenesis, sequencing, introduction of DNA into
cells and gene expression, and analysis of proteins, are
described in detail in *Current Protocols in Molecular Biology*,
Second Edition, Ausubel et al. eds., John Wiley & Sons, 1988,
15   *Short Protocols in Molecular Biology: A Compendium of Methods
from Current Protocols in Molecular Biology,* Ausubel et al.
eds., John Wiley & Sons, 4th edition 1999. The disclosures of
Sambrook et al. and Ausubel et al. (both) are incorporated
herein by reference.
20

Thus, a further aspect of the present invention provides a
host cell containing nucleic acid as disclosed herein. Such a
host cell may be *in vitro* and may be in culture. Such a host
cell may be *in vivo*. *In vivo* presence of the host cell may
25   allow intracellular expression of the specific binding members
of the present invention as "intrabodies" or intracellular
antibodies. Intrabodies may be used for gene therapy [112].

A still further aspect provides a method comprising
30   introducing such nucleic acid into a host cell. The
introduction may employ any available technique. For
eukaryotic cells, suitable techniques may include calcium
phosphate transfection, DEAE-Dextran, electroporation,
liposome-mediated transfection and transduction using

retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. Introducing nucleic acid in the host cell, in particular a eukaryotic cell may use a viral or a plasmid based system. The plasmid system may be maintained episomally

5 or may incorporated into the host cell or into an artificial chromosome [110,111]. Incorporation may be either by random or targeted integration of one or more copies at single or multiple loci. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and

10 transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

15

In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with

20 standard techniques.

The present invention also provides a method which comprises using a construct as stated above in an expression system in order to express a specific binding member or polypeptide as

25 above.

Aspects and embodiments of the present invention will now be illustrated by way of example with reference to the following experimentation.

30

EXAMPLE 1

Isolation of anti-IL-13 scFv

ScFv antibody repertoire

5   A large single chain Fv (scFv) human antibody library derived
from spleen lymphocytes from 20 donors and cloned into a
phagemid vector was used for selections [66].

Selection of scFv

10   ScFv which recognised IL-13 were isolated from phage display
libraries in a series of repeated selection cycles on
recombinant bacterially derived human or murine IL-13
(Peprotech) essentially as described in [67]. In brief,
following incubation with the library, the immobilised
15   antigen, which had been pre-coupled to paramagnetic beads, and
bound phage were recovered by magnetic separation whilst
unbound phage were washed away. Bound phage was then rescued
as described by Vaughan et al [67] and the selection process
repeated. Different solid surfaces and capture methods were
20   used at different rounds of selection to reduce non-specific
binding. Antigen was either covalently coupled to beads
(Dynabeads M-270 carboxylic acid) or modified by biotinylation
prior to secondary capture by streptavidin-coated beads
(Dynabeads M-280)   according to manufacturer's protocols
25   (Dynal). A representative proportion of clones from the output
of selection rounds were subjected to DNA sequencing as
described in Vaughan et al [67] and Osbourn et al [70].
Unique clones were assessed for their ability to neutralise
IL-13 as purified scFv preparations in IL-13 dependent cell
30   proliferation assays.

Ribosome display libraries were created and screened for scFv
that specifically recognised recombinant, bacterially derived
human or murine IL-13 (Peprotech), essentially as described in

Hanes *et al* [113]. Initially the BAK278D6 lead clone from the initial selections was converted to ribosome display format, and this template was subsequently used for library creation. On the DNA level, a T7 promoter was added at the 5'-end for

5 efficient transcription to mRNA. On the mRNA level, the construct contained a prokaryotic ribosome-binding site (Shine-Dalgarno sequence). At the 3' end of the single chain, the stop codon was removed and a portion of gIII (gene III) was added to act as a spacer [113].

10

Ribosome display libraries derived from BAK278D6 were created by mutagenesis of antibody complementarity determining regions (CDRs) where PCR reactions were performed with non-proof reading *Taq* polymerase. Affinity-based selections were

15 performed whereby, following incubation with the library, the biotinylated human-IL-13 was captured by streptavidin-coated paramagnetic beads (Dynal M280) and bound tertiary complexes (mRNA-ribosome-scFv-IL-13) were recovered by magnetic separation whilst unbound complexes were washed away. The

20 mRNA encoding the bound scFvs were then recovered by RT-PCR as described in Hanes *et al* [113] and the selection process repeated with decreasing concentrations (100nM - 100pM over 5 rounds) of biotinylated human IL-13 present during the selection.

25

Error-prone PCR was also used to further increase library size. Three intensities of error were employed (2.0, 3.5 and 7.2 mutations per 1,000 bp after a standard PCR reaction, as described in manufacturer's protocol (Clontech)) during the

30 selection regime. Initial error prone PCR reactions took place before round one selections commenced at 100nM. A subsequent round of error prone PCR was performed before round three selections at 10nM biotinylated human-IL-13. As above, a representative proportion of clones from the output of

selection rounds were subjected to DNA sequencing as described in Vaughan et al [67] and Osbourn et al [70]. Unique clones were assessed for their ability to neutralise IL-13 as purified scFv preparations in IL-13 dependent cell

5   proliferation assays.

EXAMPLE 2

Neutralisation potency of anti-IL-13 scFv in the IL-13 dependent TF-1 cell proliferation assay

10

The neutralisation potency of purified scFv preparations against human and murine IL-13 bioactivity was assessed using TF-1 cell proliferation assay. Purified scFv preparations were prepared as described in Example 3 of WO01/66754. Protein

15   concentrations of purified scFv preparations were determined using the BCA method (Pierce). TF-1 is a human premyeloid cell line established from a patient with erythroleukemia [68]. The TF-1 cell line is factor dependent for survival and proliferation. In this respect TF-1 cells responded to either

20   human or murine IL-13 [69] and were maintained in media containing human GM-CSF (4 ng/ml, R&D Systems). Inhibition of IL-13 dependent proliferation was determined by measuring the reduction in incorporation of tritiated thymidine into the newly synthesized DNA of dividing cells.

25

TF-1 cell assay protocol

TF-1 cells were obtained from R&D Systems and maintained according to supplied protocols. Assay media comprised RPMI-1640 with GLUTAMAX I (Invitrogen) containing 5% foetal bovine

30   serum (JRH) and 1% sodium pyruvate (Sigma). Prior to each assay, TF-1 cells were pelleted by centrifugation at 300 x *g* for 5 mins, the media removed by aspiration and the cells resuspended in assay media. This process was repeated twice with cells resuspended at a final concentration of 10^5 cells/ml

in assay media. Test solutions of antibody (in triplicate) were diluted to the desired concentration in assay media. An irrelevant antibody not directed at IL-13 was used as a negative control. Recombinant bacterially derived human or

5   murine IL-13 (Peprotech) was added to a final concentration of 50 ng/ml when mixed with the appropriate test antibody in a total volume of 100 µl/well in a 96 well assay plate. The concentration of IL-13 used in the assay was selected as the dose that at final assay concentration gave approximately 80%

10   of the maximal proliferative response. All samples were incubated for 30 minutes at room temperature. 100 µl of resuspended cells were then added to each assay point to give a total assay volume of 200 µl/well. Assay plates were incubated for 72 hours at 37°C under 5% CO_2. 25 µl of tritiated

15   thymidine (10 µCi/ml, NEN) was then added to each assay point and assay plates were returned to the incubator for a further 4 hours. Cells were harvested on glass fibre filter plates (Perkin Elmer) using a cell harvester. Thymidine incorporation was determined using a Packard TopCount microplate liquid

20   scintillation counter. Data were analysed using Graphpad Prism software.

Results

Despite alternating selection cycles between human and murine
antigen no cross-reactive neutralising antibodies were
obtained. Two distinct anti-human and one anti-murine IL-13

5   neutralising scFvs were obtained from selections. BAK278D6 (VH
SEQ ID NO: 13; VL SEQ ID NO: 14) and BAK167A11 (VH SEQ ID NO:
23; VL SEQ ID NO: 24) recognised human IL-13 whilst BAK209B11
(VH SEQ ID. NO: 25; VL SEQ ID NO: 26) recognised murine IL-13.
BAK278D6 (Figure 2) and BAK167A11 (Figure 1) as scFv

10   neutralised 25 ng/ml human IL-13 with an IC_{50} of 44nM and 111nM
respectively. BAK209B11 (Figure 3) as a scFv neutralised 25
ng/ml murine IL-13 with an IC_{50} of 185nM.

EXAMPLE 3

15   *Neutralisation potency of lead clones from targeted*
optimisation of heavy chain CDR3 of parental clones in the IL-
13 dependent TF-1 cell proliferation assay

Osbourn et al. [70] have demonstrated that targeted

20   mutagenesis of residues within heavy chain CDR3 can
significantly improve the affinity of antibodies. Selections
were performed as described in Example 1, on scFv repertoires
in which residues within the heavy chain CDR3 of BAK278D6 (SEQ
ID NO: 6) BAK167A11 (SEQ ID NO: 57) had been randomised by

25   mutagenesis. Unique clones from the selection output were
identified by DNA sequencing and their neutralising potency
assessed as scFv in the TF-1 cell proliferation assay, as
described in Example 2.

30   *Results*

Significant gains in potency were achieved for both lineages.
The most potent clones from the BAK167A11 lineage were
BAK615E3, BAK612B5 and BAK582F7 which as scFv had IC_{50} of 3nM
(Figure 1), 6.6nM, 6.65nM respectively against 25ng/ml human

IL-13 in TF-1 cell proliferation assay. From the BAK278D6
lineage, the most potent clone was BAK502G9, which as scFv had
IC_{50} of 8nM against 25 ng/ml human IL-13 in the TF-1 cell
proliferation assay (Figure 2).

5

EXAMPLE 4
*Neutralisation potency of BAK167A11 and BAK278D6 lineages
against non-human primate IL-13 and an IL-13 variant
associated with asthma in the TF-1 factor dependent cell*
10 *proliferation assay*

Neither of the BAK167A11 and BAK278D6 human IL-13 neutralising
lineages were murine cross-reactive. The inventors therefore
decided on the following criteria for the lineage selected for
15 further optimisation and clinical development: should
preferably be cross-reactive with non-human primate IL-13 and
should recognise a variant of IL-13, in which arginine at
amino acid at position 130 is substituted for by glutamine
(Q130R). This variant has been genetically associated with
20 asthma and other allergic diseases [37, 39, 41, 71]. Cross-
reactivity was determined by the ability of purified scFv
preparations to bind non-human primate IL-13 and IL-13 variant
by surface plasmon resonance (BIAcore) analysis. Functional
activity was determined using the TF-1 cell proliferation
25 assay.

Production of wild-type, variant and non-human primate IL-13
A cDNA for wild-type human IL-13 was obtained from InvivoGen
and modified by site-directed mutagenesis (Stratagene
30 Quikchange® kit) to yield a cDNA encoding variant IL-13. The
coding sequence for both rhesus and cynomolgus monkey IL-13
was obtained by PCR on genomic DNA template using degenerate
primers based on the human IL-13 sequence. Both non-human
primate (rhesus and cynomolgus) sequences were identical to

each other but differed from human IL-13 by seven amino acids (Figure 19). Recombinant wild type, variant and non-human primate IL-13 were subsequently expressed using the baculovirus expression system (Invitrogen). Expression

5 constructs added a carboxyl terminus affinity tag to the expressed protein that allowed purification from insect cell conditioned media to near homogeneity.

Qualitative binding assay using BIAcore

10 The binding affinity of purified scFv preparations to non-human primate, variant and wild type IL-13 was determined by surface plasmon resonance measurements using a BIAcore 2000 Biosensor (BIAcore AB) as described in Karlsson et al [72]. In brief, IL-13 was coupled to CM5 sensorchips using an amine

15 coupling kit (BIAcore) at a surface density of approximately 200Ru and three concentrations of test scFv (approximately 350nM, 175nM and 88nM) in HBS-EP buffer passed over the sensor chip surface. The resulting sensorgrams were evaluated using BIA evaluation 3.1 software to provide relative binding data.

20

TF-1 assay protocol

The assay was performed essentially as described in Example 2 with the following modifications: non-human primate IL-13,

25 human variant IL-13 (130R) and wild type human IL-13 were used at concentrations of 50 ng/ml, 25 ng/ml and 25 ng/ml respectively.

Results

30 BIAcore binding assay data suggested that BAK278D6 but not BAK167A11 lineage had the required cross-reactivity profile for further therapeutic development (Table 2). This finding was supported by bioassay data demonstrating that BAK278D6 (Figure 4) and BAK502G9 (Figure 6) were able to neutralise

human IL-13, the human IL-13 (Q130R) variant and non-human
primate IL-13 in the TF-1 cell proliferation assay with near
equivalent potency. In contrast, although BAK615E3 (VH SEQ ID
NO: 33; VL SEQ ID NO: 34) had a significantly increased
5   potency against human IL-13 over its parent BAK167A11 (VH SEQ
ID NO: 23; VL SEQ ID NO: 24) in the TF-1 cell proliferation
assay (Figure 1), neither clone bound non-human primate or
variant IL-13 in the BIAcore binding assay.

10 *Germlining framework regions of BAK278D6 and BAK502G9*
The derived amino acid sequence of BAK278D6 VH (SEQ ID NO: 13)
and VL (SEQ ID NO: 14) were aligned to the known human
germline sequences in the VBASE database [73] and the closest
germline identified by sequence similarity. The closest
15 germline for the VH domain of BAK278D6 (SEQ ID NO: 14) and its
derivatives, was identified as DP14, a member of the VH1
family. The BAK278D6 VH has 9 changes from the DP14 germline
within framework regions. The closest germline for the VL of
BAK278D6 was identified as $V_\lambda 3$ 3h. The BAK278D6 VL domain (SEQ
20 ID NO: 14) has only 5 changes from the germline within
framework regions. Framework regions of BAK278D6 and its
derivatives were returned to germline by site directed
mutagenesis (Stratagene Quikchange kit) to identically match
native human antibodies.
25

EXAMPLE 5
Neutralisation potency of lead clones from targeted
optimisation of heavy chain CDR1 and heavy chain CDR2
sequences of BAK502G9 in the human IL-13 dependent TF-1 cell
30 *proliferation assay*

A second phase of optimisation was performed using BAK502G9
sequence, with germlined framework regions, as a template.
Selections were performed essentially as described in Example

1 on scFv repertories in which either residues within the
heavy chain CDR1 or heavy chain CDR2 of BAK502G9 had been
randomised by mutagenesis. Unique clones from the selection
output were identified by DNA sequencing and their

5 neutralising potency assessed as purified scFv preparations in
the TF-1 cell proliferation assay as described in Example 2.
Vectors were constructed for the most potent scFv clones to
allow re-expression as whole human IgG4 antibody as described
by Persic et al. (1997 Gene 187; 9-18) with a few

10 modifications. An oriP fragment was included in the vectors to
facilitate use with HEK-EBNA 293 cells and to allow episomal
replication. The VH variable domain was cloned into the
polylinker between the secretion leader sequence and the human
gamma 4 constant domain of the expression vector pEU8.1(+).

15 The VL variable domain was cloned into the polylinker between
the secretion leader sequence and the human lambda constant
domain of the expression vector pEU4.1(-).

Whole antibody was purified from conditioned media from EBNA-
20 293 cells co-transfected with constructs expressing heavy and
light chains by protein A affinity chromatography (Amersham
Pharmacia). The purified antibody preparations were sterile
filtered and stored at 4°C in phosphate buffered saline (PBS)
prior to evaluation. Protein concentration was determined by

25 measuring absorbance at 280nm using the BCA method (Pierce).
Reformatted human IgG4 whole antibodies were compared to
commercially available anti-human IL-13 antibodies in the TF-1
proliferation assay described in Example 2.

30 *Results*
As demonstrated in Figure 5, the commercial antibody B-B13,
(mouse IgG1 -Euroclone 5) was shown to be significantly more
potent against human IL-13 than the commercial antibody JES10-
5A2 (rat IgG1 - Biosource) with IC_{50} of 1021pM and 471pM

respectively. Eight clones, namely, BAK1111D10, BAK1166G02, BAK1167F02, BAK1167F04, BAK1183H4, BAK1184C8, BAK1185E1, BAK1185F8, derived from BAK502G9 (and so "BAK502G9 lineage"), in which the heavy chain CDR1 or CDR2 had been targeted,

5   showed improved potency as scFv over the commercial antibodies. These improvements were maintained on conversion to whole antibody human IgG4. Each of these VH and VL domains individually and in the respective pairings of these claims represents an aspect or embodiment of the present invention,

10   as do specific binding members for IL-13 that comprise one or more of them, also specific binding members comprising one or more CDR's from the BAK502G9 lineage clones, preferably a VH domain comprising a BAK502G9 lineage set of HCDR's and/or a VL domain comprising a BAK502G9 lineage set of LCDR's. These may

15   be employed in any and all aspects of the invention as disclosed elsewhere herein. Derivatives of BAK502G9 as whole antibodies (IgG4) had an IC_{50} ranging from 244pM to 283pM. BAK502G9 as a whole antibody IgG4 had an IC_{50} of 384pM. In summary, major improvements in potency could be obtained by

20   targeting heavy chain CDR1 (SEQ ID NO:7) or CDR2 (SEQ ID NO: 8) of BAK502G9. Statistical comparisons to B-B13 were made using an ANOVA followed by a Dunnett's post test analysis (InStat software).

25   *Further characterisation*
       Selected anti-human antibodies from the BAK278D6 lineage underwent further characterisation to determine their specificity. These included BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16) and its derivatives BAK1167F2 (VH SEQ ID NO: 35; VL

30   SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), which are representative examples of clones with modifications to heavy chain CDR1 and heavy chain CDR2 of BAK502G9 respectively.

EXAMPLE 6

Neutralisation potency of lead clones from targeted
optimisation of heavy chain CDR1 and heavy chain CDR2
sequences of BAK502G9 against non-human primate IL-13 and an

5 *IL-13 variant associated with asthma in the TF-1 factor*
dependent cell proliferation assay

Cross-reactivity of anti-human IL-13 antibodies was determined
by their ability to inhibit non-human primate IL-13 and IL-13

10 variant mediated TF-1 cell proliferation as described in
Example 4.

Results

Optimised anti-human IL-13 antibodies BAK1167F2 (VH SEQ ID NO:

15 35; VL SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ
ID NO: 38) maintained the specificity of their parent BAK502G9
(VH SEQ ID NO: 15; VL SEQ ID NO: 16) (Figure 6). Potency gains
against wild type IL-13 were reflected in their ability to
neutralise non-human primate IL-13 and an IL-13 variant with

20 equivalent potency. The IC_{50} for BAK502G9 against human, human
variant and non-human primate IL-13 were 1.4nM, 1.9nM and
2.0nM respectively. The IC_{50} for BAK1167F2 against human, human
variant and non-human primate IL-13 were 1.0nM, 1.1nM and
1.3nM respectively. The IC_{50} for BAK1183H4 against human, human

25 variant and non-human primate IL-13 were 0.9nM, 1.0nM and
1.6nM respectively. These clones are suitable for therapeutic
use.

EXAMPLE 7

30 *Neutralising potency of lead anti-human IL-13 antibodies*
against native human IL-13 in HDLM-2 cell proliferation assay

The human IL-13 sequence has four potential N-glycosylation
sites. The inventors have demonstrated the ability of BAK278D6

and its derivatives to neutralise recombinant IL-13 expressed
either in bacterial or baculovirus expression systems.
Although, there is evidence that many processing events known
in mammalian systems do also occur in insects there are key
5   differences in protein glycosylation, particularly N-
glycosylation [74].

The inventors investigated the ability of BAK278D6 derivatives
to neutralise native IL-13 released from human cells.
10

HDLM-2 cells were isolated by Drexler et al [75] from a
patient with Hodgkin's disease. Skinnider et al [76]
demonstrated that HDLM-2 cell proliferation was in part
dependent on autocrine and paracrine release of IL-13. Lead
15   anti-human IL-13 antibodies were assessed for their ability to
inhibit HDLM-2 cell proliferation mediated by the release of
native IL-13.

HDLM-2 cell assay protocol
20   HDLM-2 cells were obtained from the Deutsche Sammlung von
Mikroorganismen und Zellkulturen (DSMZ) and maintained
according to supplied protocols. Assay media comprised RPI-
1640 with Glutamax I (Invitrogen) containing 20% foetal bovine
serum. Prior to each assay, the cells were pelleted by
25   centrifugation at 300x g for 5 min, the media removed by
aspiration and the cells resuspended in fresh media. This
process was repeated three times and the cells were finally
resuspended to a final concentration of 2×10^5 cells/ml in
assay media. 50µl of resuspended cells were added to each
30   assay point in a 96 well assay plate. Test solutions of
antibodies (in triplicate) were diluted to the desired
concentration in assay media. An irrelevant isotype antibody
not directed at IL-13 was used as a negative control. The
appropriate test antibody in a total volume of 50µl / well

were added to the cells, each assay point giving a total assay volume of 100µl / well. Assay plates were incubated for 72 hours at 37°C under 5% CO_2. 25 µl of tritiated thymidine (10 µCi/ml, NEN) was then added to each assay point and assay

5   plates were returned to the incubator for a further 4 hours. Cells were harvested on glass fibre filter plates (Perkin Elmer) using a cell harvester. Thymidine incorporation was determined using a Packard TopCount microplate liquid scintillation counter. Data were analysed using Graphpad Prism

10 software.

Results

As demonstrated in Figure 7, BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), and its derivatives BAK1183H4 (VH SEQ ID NO:

15 37; VL SEQ ID NO: 38) and BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) were able to cause a dose dependent inhibition of cell proliferation with relative potencies similar to those observed in other bioassays. IC_{50} for BAK502G9, BAK1183H4, BAK1167F2 as human IgG4 were 4.6nM, 3.5nM and 1.1nM

20 respectively. IC_{50} for the commercial antibodies JES10-5A2 and B-B13 were 10.7nM and 16.7nM respectively.

EXAMPLE 8

Neutralising potency of lead anti-human IL-13 antibodies

25 *against IL-13 dependent responses in disease relevant primary cells*

Secondary bioassays were performed using primary cells and readouts more relevant to airway disease. These included

30 eotaxin release from normal human lung fibroblasts (NHLF) and vascular adhesion molecule 1 (VCAM-1) upregulation on the surface of human umbilical vein endothelial cells (HUVEC). Both IL-13 dependent responses could contribute to eosinophil recruitment, a feature of the asthma phenotype [92].

NHLF assay protocol

IL-13 has been shown to cause eotaxin release from lung
fibroblasts[77] [78] [79]. Factor dependent eotaxin release
5   from NHLF was determined by ELISA.

NHLF were obtained from Biowhittaker and maintained according
to supplied protocols. Assay media was FGM-2 (Biowhittaker).
Test solutions of antibody (in triplicate) were diluted to the
10   desired concentration in assay media. An irrelevant antibody
not directed at IL-13 was used as a negative control.
Recombinant bacterially-derived human IL-13 (Peprotech) was
subsequently added to a final concentration of 10 ng/ml when
mixed with the appropriate test antibody in a total volume of
15   200 µl. The concentration of IL-13 used in the assay was
selected as the dose that gave an approximately 80% of the
maximal response. All samples were incubated for 30 minutes at
room temperature. Assay samples were then added to NHLF that
had been preseeded at a density of 1×10^4 cells per well in
20   96-well assay plates. Assay plates were incubated at 37°C for
16-24 hours at 37°C under 5% CO_2. Assay plates were centrifuged
at 300 x *g* for 5 minutes to pellet detached cells. Eotaxin
levels in the supernatant were determined by ELISA using
reagents and methods described by the manufacturer (R&D
25   Systems). Data were analysed using Graphpad Prism software.

Results

BAK278D6 lineage clones were able to inhibit human IL-13
dependent eotaxin release from NHLF. Relative potency was
30   similar to that observed in the TF-1 cell proliferation assay
(Figure 8). BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16),
BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), BAK1167F2 (VH
SEQ ID NO: 35; VL SEQ ID NO: 36) had IC_{50} of 207pM, 118pM and
69pM respectively against 10 ng/ml human IL-13. Commercial

antibodies JES10-5A2 and B-B13 had IC_{50} of 623pM and 219pM respectively.

HUVEC assay protocol

5   IL-13 has been shown to upregulate expression of VCAM-1 on
·   cell surface of HUVECs [80, 81]. Factor dependent VCAM-1
     expression was determined by detection of upregulation of
     VCAM-1 receptor cellular expression using a time-resolved
     fluorescence read out.
10
     HUVEC were obtained from Biowhittaker and maintained according
     to supplied protocols. Assay media was EGM-2 (Biowhittaker).
     Test solutions of antibody (in triplicate) were diluted to the
     desired concentration in assay media. An irrelevant antibody
15   not directed at IL-13 was used as a negative control.
     Recombinant bacterially derived human IL-13 (Peprotech) was
     added to a final concentration of 10 ng/ml when mixed with the
     appropriate test antibody in a total volume of 200 µl. The
     concentration of IL-13 used in the assay was selected as the
20   dose that gave approximately 80% of the maximal response. All
     samples were incubated for 30 minutes at room temperature.
     Assay samples were then added to HUVEC that had been preseeded
     at 4×10^4 cells per well in 96-well assay plates. Assay
     plates were incubated at 37°C for 16-20 hours under 5% CO_2.
25   Assay media was then removed by aspiration and replaced with
     blocking solution (PBS containing 4% dried Marvel® milk
     powder). Assay plates were incubated at room temperature for 1
     hour at room temperature. Wells were washed three times with
     PBST Tween before 100 µl (1:500 dilution in PBST/1% Marvel®)
30   of biotinylated anti-VCAM-1 antibody (Serotec) was added to
     each well. Assay plates were incubated at room temperature for
     1 hour. Wells were washed three times with Delfia wash buffer
     (Perkin Elmer) before 100 µl of Europium-labelled Streptavidin
     or anti-murine IgG1 (1:1000 dilution in Delfia assay buffer,

Perkin Elmer) was added to each well. Assay plates were then incubated at RT for 1 hour. Wells were washed 7 times with Delfia wash buffer (Perkin Elmer). Finally, 100µl of enhancement solution (Perkin Elmer) was added to each well and

5   fluorescence intensity was determined using the Wallac 1420 VICTOR2 plate reader (Standard Europium protocol). Data were analysed using Graphpad Prism software.

Results

10   Typical data for BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) as whole antibody human IgG4 are shown in Figure 9. Relative potency was similar to the observed in the TF-1 cell proliferation assay. IC_{50} for

15   BAK502G9, BAK1183H4 and BAK1167F2 were 235pM, 58pM and 55pM respectively against 10ng/ml human IL-13.

EXAMPLE 9

Neutralisation potency of anti-IL-13 antibodies against IL-1β

20   *and IL-4 dependent VCAM-1 upregulation*

The specificity of the BAK278D6 lineage of clones was assessed in a modification of the HUVEC bioassay. Together with IL-13, both IL-4 and IL-1β have been shown to upregulate expression

25   of VCAM-1 on cell surface of HUVECs [80, 81].

HUVEC assay protocol

The assay was performed essentially as described in Example 5 with the following modifications. Recombinant human IL-1β and

30   IL-4 (R&D Systems) were used in place of human IL-13 at 0.5 ng/ml and 1 ng/ml respectively and represented the dose that gave approximately 80% of the maximal response.

Results

None of the clones evaluated from the BAK278D6 lineage neutralised VCAM-1 upregulation in response to either human IL-1β or IL-4 and thus demonstrated specificity for IL-13

5 (Figure 10). IL-4 is most closely related to IL-13, sharing 30% sequence identity at the amino acid level [82].

EXAMPLE 10

Neutralisation potency of BAK209B11 as a human IgG4 in a

10 *murine IL-13 dependent murine B9 cell proliferation assay*

BAK209B11, identified as an anti-murine IL-13 neutralising clone as a scFv as described in Example 1, was reformatted as a whole antibody human IgG4 as described in Example 5 and its

15 potency evaluated in the murine IL-13 dependent B9 cell proliferation assay. B9 is a murine B-cell hybridoma cell line [83]. B9 is factor dependent for survival and proliferation. In this respect B cells respond to murine IL-13 and are maintained in media containing human IL-6 (50pg/ml, R&D

20 Systems). Inhibition of murine IL-13 dependent proliferation was determined by measuring the reduction in incorporation of tritiated thymidine into the newly synthesized DNA of dividing cells.

25 *B9 cell assay protocol*

B9 cells were obtained from European Collection of Animal Cell Culture ECACC and maintained according to supplied protocols. The assay was performed essentially as described for the TF-1 assay in Example 2 but with the following modifications. Assay

30 media comprised RPMI-1640 with GLUTAMAX I (Invitrogen) containing 5% foetal bovine serum (Hyclone) and 50μM 2-mercaptoethanol (Invitrogen). Recombinant bacterially derived murine IL-13 (Peprotech) replaced human IL-13 with a final assay concentration of 1ng/ml.

Results

BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) as a human
IgG4 neutralised 1 ng/ml murine IL-13 with an IC_{50} of 776pM in
the B9 assay (Figure 11). BAK209B11 therefore represents a
useful tool to investigate the role of IL-13 in murine models
of disease. This is clearly demonstrated in Example 12, which
demonstrates the efficacy of BAK209B11 in a murine model of
acute pulmonary inflammation.

EXAMPLE 11

*Affinity determination of anti-IL-13 antibodies by BIAcore
analysis*

The affinity of BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16),
BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) and BAK1183H4
(VH SEQ ID NO: 37; VL SEQ ID NO: 38) for human IL-13 and
BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) for murine IL-
13 as human IgG4 were determined by surface plasmon resonance
measurements using a BIAcore 2000 Biosensor (BIAcore AB)
essentially as described in [72]. In brief, antibodies were
coupled to CM5 sensorchips using an amine coupling kit
(BIAcore) at a surface density of approximately 500Ru and a
serial dilution of IL-13 (between 50nM to 0.78nM) in HBS-EP
buffer was passed over the sensorchip surface. The resulting
sensorgrams were evaluated using BIA evaluation 3.1 software
to provide kinetic data.

Results

BAK502G9, BAK1167F2 and BAK1183H4 IgG4 bound human IL-13 with
high affinity with Kd of 165pM, 136pM and 81pM respectively
corresponding to their relative potency in cell based assays.
BAK209B11 bound murine IL-13 with affinity of 5.1nM   (Table
3).

EXAMPLE 12

Efficacy of BAK209B11 in a murine model of acute allergic pulmonary inflammation

5

Murine model of acute allergic pulmonary inflammation

The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti-murine IL-13 neutralising human IgG4 antibody, was investigated in a murine of acute allergic pulmonary

10 inflammation. This model was performed essentially as described by Riffo-Vasquez et al [84] and is characterised at its endpoint by increased bronchial alveolar lavage (BAL) IL-13 (Figure 12), cellular infiltration into the lung and BAL (Figure 13), increased serum IgE levels and airways

15 hyperresponsiveness (AHR).

Model protocol

Female Balb/C mice (Charles River UK) were treated with either anti-murine IL-13 antibody BAK209B11 (at 12, 36, 119 or 357 μg

20 doses) or an isotype matched control antibody (357 μg dose). On days 0 and 7, mice in each group were sensitised by intraperitoneal injection of 10μg of ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2% Al_2O_3 (Rehydragel) as an adjuvant). A separate control group of non-sensitised mice

25 received an equal volume of the vehicle. Mice were challenged with ovalbumin on days 14, 15 and 16. Ovalbumin was diluted to 1% (w/v) in sterile saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss

30 Ultraneb 2000 nebuliser (Sunrise Medical) in a series of three exposures of 20 minutes separated by 1 hour intervals.

BAK209B11 or an irrelevant human IgG4 were administered intravenously, 1 day prior to first challenge and then 2 hours

prior to each subsequent challenge (4 doses in total). The
model ended at day 17, 24 hours post final challenge. Blood
(serum) and BAL were collected. Serum was assayed for total
IgE. BAL was obtained by injecting 3 aliquots of saline
5   (0.3ml, 0.3ml and 0.4ml) and pooling samples. Total leukocytes
and differential cell counts were obtained from BAL cells.

Results
Ovalbumin challenge of sensitised mice caused a significant
10   ($p<0.05$) increase in total BAL cell recruitment over non-
sensitised but challenged animals. This recruitment was dose-
dependently inhibited by BAK209B11; significant ($p<0.05$)
inhibition was seen with $\geq 36\mu g$ BAK209B11, but not control
antibody (Figure 13). Similar effects were also seen on
15   eosinophils (Figure 14) and neutrophils (Figure 15) with
significant ($p<0.05$) inhibition of cellular influx at a
minimum BAK209B11 dose of $36\mu g$. This inhibition was not seen
with the control antibody. Lymphocytes were also induced in
sensitised but not non-sensitised mice upon challenge. This
20   induction was dose-dependently inhibited by BAK209B11, with
maximal inhibition seen with $36\mu g$ BAK209B11. Control antibody
had no effect (Figure 16). Although monocyte/macrophages were
not induced in sensitised animals when compared to non-
sensitised animals, background levels were depressed by $\geq 36\mu g$
25   BAK209B11, but not by control antibody (Figure 17). Serum IgE
levels were significantly increased in sensitised animals when
compared to non-sensitised after challenge ($p<0.05$). This
increase was decreased after treatment with $36\mu g$ BAK209B11 but
not by the control antibody.
30

In summary, systemic administration of BAK209B11, a murine IL-
13 neutralising antibody, but not control antibody inhibited
inflammatory cell influx and the upregulation of serum IgE

levels caused by sensitisation and subsequent challenge with ovalbumin in a murine model of allergic inflammation.

Examples 13 to 20 are prophetic.

5

EXAMPLE 13
Efficacy of BAK209B11 in the Lloyd murine model of acute pulmonary inflammation

10 *Murine model of acute allergic pulmonary inflammation*
The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti murine IL-13 neutralising antibody, was investigated in a second murine model of acute allergic pulmonary inflammation. This model was performed essentially as
15 described by McMillan et al. [85] and is characterised at its endpoint by increased BAL and lung tissue IL-13, cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

20 *Model protocol*
Female Balb/C mice (Charles River UK) were administered with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody, as follows. On days 0 and 12, mice in each group were sensitised (SN) by intraperitoneal
25 injection of 10µg of ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2mg Al(OH)$_3$ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle. Mice were challenged with ovalbumin for 20 minutes on days 19,
30 20, 21, 22, 23 and 24. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise Medical). On days 18,19,20,21,22,23 and 24 mice were

administered with various intraperitoneal doses (237µg, 23.7µg or 2.37µg; denoted in figure 21 by H,M and L) of anti-murine IL-13 antibody BAK209B11 muIgG1 or an isotype matched control antibody (237µg). Airway function was assessed on days 0 and

5 25 by increasing methacholine challenges and monitored using conscious plethysmography (Buxco). PC_{50} (concentration of methacholine required to increase baseline PenH by 50%) was estimated for individual mice at both day 0 and day 25 from 4 parameter unfixed curve fitting of methacholine dose-response

10 curves.

The model ended at day 25, 24 hours post final challenge. Blood, serum, BAL and lung tissue were collected.

15 *Results*

Lung function was evaluated for individual animals at day 0 (pre-treatment) and at day 25 (post-challenge) and was quantitated by calculating PC_{50} values (concentration of methacholine required to increase baseline PenH by 50%)

20 (Figure 21A). An individuals airways hyperresponsiveness (AHR) was determined by the change in log PC_{50} at day 25 versus day 0 (log day 25 PC_{50} - log day 0 PC_{50}). This delta $logPC_{50}$ was the primary endpoint of the study; PC_{50} data log-transformed because of requirements of endpoint ANOVA.

25 Individual changes were averaged within groups to generate group average delta log PC_{50} (as shown in Figure 21B).

Ovalbumin challenge of sensitised mice caused a significant AHR compared to non-sensitised and challenged mice (p<0.01).

30 BAK209B11 caused a clear and dose-dependent decrease in AHR whereas the control antibody had no effect.

EXAMPLE 14

Efficacy of BAK209B11 in the Gerard murine model of acute pulmonary inflammation

5

Murine model of acute allergic pulmonary inflammation
The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti-murine IL-13 neutralising human IgG4 antibody, was investigated in a third murine model of acute allergic
10 pulmonary inflammation. This model was performed essentially as described by Humbles *et al.* [86] and is characterised at its endpoint by increased BAL and lung tissue IL-13, cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).
15

Model protocol
Female Balb/C mice (Charles River UK) were administered with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody. On days 0, 7 and 14, mice in
20 each group were sensitised (SN) by intraperitoneal injection of 10µg of ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 1.125mg Al(OH)$_3$ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle.
25 Mice were challenged with ovalbumin for 20 minutes on days 21, 22, 23 and 24. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise
30 Medical).

The model ended at day 25, 24 hours post challenge. Blood, serum, BAL and lung tissue were collected.

EXAMPLE 15

Efficacy of BAK209B11 in the Lloyd chronic model of pulmonary inflammation

5 *Murine model of chronic allergic pulmonary inflammation*
The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti murine IL-13 neutralising human IgG4 antibody, was investigated in a model of chronic allergic pulmonary inflammation. This model was performed essentially as
10 described by Temelkovski *et al.* [87] and is characterised at its endpoint by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

15 *Model protocol*
Female Balb/C mice (Charles River UK) were dosed with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody. On days 0 and 11, mice in each group were sensitised (SN) by intraperitoneal injection of 10µg of
20 ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2mg Al(OH)$_3$ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle. Mice were challenged with ovalbumin for 20 minutes on days 18, 19, 20, 21, 22, 23,
25 28, 30, 32, 35, 37, 39, 42, 44, 46, 49 and 51. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise Medical).
30

The model ended at day 52, 24 hours post challenge. Blood, serum, BAL and lung tissue were collected.

EXAMPLE 16

Efficacy of anti-human IL-13 antibodies against exogenous
human IL-13 administered to the murine air pouch model

The effect of anti-human IL-13 antibodies on the pro-
5   inflammatory action of human IL-13 was investigated in a basic
murine model. This model was performed essentially as
described by Edwards et al [93] and was characterised at its
endpoint by cellular infiltration into the airpouch.

10 *Model protocol*
An air pouch was created on the back of female Balb/C mice by
subcutaneous injection of 2.5mL of sterile air at day 0.   The
air pouch was reinflated with another 2.5mL sterile air at day
3.   2µg huIL-13 in 0.75% CMC was injected directly into the
15 pouch at day 6.   24 hours later the mice were killed and the
air pouch lavaged with 1mL heparinised saline.   Antibody
treatments were either given with the huIL-13 (into the pouch)
or given systemically.

20 *Results*
Human IL-13, injected into the airpouch (i.po.), caused a
significantly increased infiltration of total leukocytes
(p<0.01) and eosinophils (p<0.01) at 24 hours post-challenge
versus vehicle (0.75% carboxymethyl cellulose (CMC) in saline
25 i.po.) treated mice.

Locally administered BAK502G9 (200mg, 20mg or 2mg intrapouch)
significantly and dose-dependently inhibited the total
leukocyte (p<0.01) and eosinophil· (p<0.01) infiltration into
30 the air pouch caused by 2µg huIL-13 in 0.75% CMC.

Systemically administered BAK209B11 (30mg/kg, 10mg/kg and
1mg/kg) also signficantly and dose-dependently inhibited the

total leukocyte (p<0.01) and eosinophil (p<0.01) infiltration
into the air pouch caused by 2μg huIL-13 in 0.75% CMC.

EXAMPLE 17

5   *Generation of human IL-13 knock-in / murine IL-13 knock out
transgenic mice for the purposes of evaluating the efficacy of
anti-human IL-13 antibodies in models of pulmonary allergic
inflammation*

10 The present inventors have generated mice which express human,
rather than murine IL-13 by gene targeting. The mouse IL-13
gene has been replaced from start to stop codon with the
relevant portion of the human IL-13 gene. This mouse strain
expresses human IL-13, rather than mouse IL-13, in response to
15 the same stimuli as in the wild-type mouse, as the endogenous
IL-13 promoter and IL-13 pA tail remaining unchanged. It has
been shown that human IL-13 can bind to and signal through
mouse IL-13 receptors to generate the same physiological
consequences as signalling caused by mouse IL-13 ligating
20 mouse IL-13 receptors. For example exogenous human IL-13
caused inflammatory cell recruitment into the murine air pouch
(Figure 18). These transgenic animals allow us to evaluate
non-murine cross reactive anti-human IL-13 antibodies in
established murine models of disease.
25

This mouse has been used in the acute allergic airway
inflammation models (as described in examples 18 and 19) and
chronic allergic airway inflammation models (as described in
Example 20) allowing the evaluation of anti-human IL-13
30 antibody pharmacology in allergic airway disease.

EXAMPLE 18

*Efficacy of anti-human IL-13 antibodies in the huIL-13-
transgenic Lloyd murine model of acute pulmonary inflammation*

Murine model of acute allergic pulmonary inflammation

The effect of anti human IL-13 neutralising human IgG4
antibodies were investigated in a murine model of acute
5   allergic pulmonary inflammation using the transgenic mice
generated in example 17. This model was performed essentially
as described by McMillan *et al.* [85] and example 13. The
model was characterised at its endpoint by increased BAL and
lung tissue IL-13, cellular infiltration into the lung and
10   BAL, increased serum IgE levels and airways
hyperresponsiveness (AHR).

Model protocol

The protocol for this model was as described in Example 13
15   except that anti-human IL-13 antibodies were dosed instead of
BAK209B11.

EXAMPLE 19

Efficacy of anti-human IL-13 antibodies in the huIL-13-
20   *transgenic Gerard murine model of acute pulmonary inflammation*

Murine model of acute allergic pulmonary inflammation

The effect of anti human IL-13 neutralising human IgG4
25   antibodies were investigated in another murine model of acute
allergic pulmonary inflammation using the transgenic mice
generated in example 17. This model was performed essentially
as described by Humbles et al, [86] and in example 14. The
model is characterised at its endpoint by increased BAL and
30   lung tissue IL-13, cellular infiltration into the lung and
BAL, increased serum IgE levels and airways
hyperresponsiveness (AHR).

Model protocol

The protocol for this model was as described in Example 14 except that anti-human IL-13 antibodies were dosed instead of BAK209B11.

5

EXAMPLE 20

Efficacy of anti-human IL-13 antibodies in the huIL-13-transgenic Lloyd chronic model of pulmonary inflammation

10 The effect of anti human IL-13 neutralising human IgG4 antibodies were investigated in a model of chronic allergic pulmonary inflammation using the transgenic mice generated in example 17. This model was performed essentially as described by Temelkovski *et al.* [87] and in Example 15 and is

15 characterised at its endpoint by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

Model protocol

20 The protocol for this model was as described in Example 15 except that anti-human IL-13 antibodies were dosed instead of BAK209B11

EXAMPLE 21

25 *Pharmacokinetics and pharmacodynamics of anti-human IL-13 antibodies in Ascaris.suum-allergic cynomolgus monkeys*

The pharmacokinetics and pharmacodynamics of 502G9 were evaluated in 4 allergic but non-challenged cynomolgus primates

30 (2 male/2 female) after a single 10mg/kg i.v bolus dose. The experiment ran for 29 days. The antibody's pharmacokinetic parameters were determined from a geomean average serum-drug concentration curve and are detailed below in Table 4.

In the same study serum IgE concentrations were also followed using a human IgE ELISA kit (Bethyl laboratories, USA).

Results

5   Serum IgE concentrations were significantly reduced after a single 10mg/kg i.v bolus dose of BAK502G9, from 100 % control levels (predose) to 66 ± 10% of control values (p<0.05), at 4 and 5 days after dosing. This lowering of serum IgE concentration recovered to 88 ± 8 % of control levels by day

10 22 (see Figure 20). Again these data were derived by normalising each animals serum IgE concentration to predose levels, where predose concentrations was 100%, and then averaging the curves from the 4 animals tested.

15 *EXAMPLE 22*
Efficacy of anti-human IL-13 antibodies in cynomolgus models of dermal allergy

The effects of anti-human IL-13 neutralising human IgG4

20 antibodies were investigated in a primate model of acute allergic dermal inflammation. This model was performed by injecting human IL-13 and *A.suum* antigen intradermally into cynomolgus monkeys. 24-96h later, dermal biopsies and serum samples were taken. The model was characterised at its

25 endpoint by cellular infiltration into the skin.

EXAMPLE 23
Efficacy of anti-human IL-13 antibodies in cynomolgus models of pulmonary allergy

30

The effect of anti human IL-13 neutralising human IgG4 antibodies were investigated in a primate model of acute allergic pulmonary inflammation. This model was performed by exposing *a.suum*-allergic cynomolgus primates to nebulised

a.suum antigen, thereby generating an allergic reaction. This allergy was characterized at its end point by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness.

5

Pharmacodynamics were additionally evaluated *ex vivo* using a flow cytometric method. CD23 is the high affinity IgE receptor and can be expressed on peripheral human blood mononuclear cells. CD23 expression can be induced, in terms 10 of the number of cells expressing CD23 and also in how much CD23 each cell expresses by both IL-13 and IL-4. The IL-13, but not IL-4, mediated response can be inhibited by anti-human IL-13 antibodies.

15 Blood, serum, BAL and lung tissue were colleted. Serum IgE levels were monitored by ELISA. Serum from BAK502G9 treated cynomolgus monkeys was shown to inhibit the expression of CD23 on human peripheral blood mononuclear cells induced by IL-13 but not IL-4. The magnitude of this inhibition was consistent 20 with the serum BAK502G9 levels predicted by PK ELISA.

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Table 1

Kabat numbering		HCDR1	HCDR2	HCDR3	linker	LCDR1	LCDR2	LCDR3	Atom TF1 (kd) appar	TF1
BAK278D6									4	
BAK502G9	PD								0	2
BAK1187B4	PD								0.1	2
BAK1187F2	PD								0.2	3
BAK1105H3	PD								0.2	2
BAK1185E1	PD								0.2	2
BAK1111D10	PD								0.2	2
BAK1184G5	PD								0.2	2
BAK1186G2	PD								0.2	2
BAK1184C8	PD								0.2	2
BAK1185F8	PD								0.2	2
BAK1187F4	PD								0.2	2
BAK1109G6	PD								0.3	3
BAK1183H4	PD								0.3	2
BAK1183D2	PD								0.3	2
BAK1184F9	PD								0.3	2
BAK1103G8	PD								0.3	2
BAK1157D08	PD								0.3	2
BAK1183B5	PD								0.4	2
BAK1097H08	PD								0.4	2
BAK1105F04	PD								0.5	2
BAK1183G5	PD								0.5	1
BAK1161H07	PD								0.8	1
BAK1162G04	PD								0.8	1
BAK1181D07	PD								0.9	1
BAK1182D09	PD								0.9	1
BAK1109F06	PD								14	1
BAK1107F08	PD								15	1
ALA VL26L	O								25	1
ALA VL26M	O								25	1
ALA VL26C	O								29	1
ALA VL26V	O								31	1
ALA VL26K	O								35	1
ALA VL26Y	O								38	1
ALA VL26F	O								39	1
ALA VL26R	O								43	1
ALA VL26T	O								45	1
BAK1001C10	RD								52	2
BAK1018G7	RD								53	2
BAK1008C3	RD								53	2
BAK1009A4	RD								54	1
BAK1010D2	RD								64	2
BAK1007F9	RD								69	1
BAK1016H9	RD								69	1
BAK1009D2	RD								72	1
ALA VL26A	O								7.5	2
BAK1007C4	RD								7.7	1
ALA VL26H	O								8.3	2
BAK1054C8	RD								9.5	1
BAK1005G7	RD								9.0	1
BAK1016F8	RD								10.0	1
BAK1063D10	RD								10.0	1
BAK1080F8	RD								11.7	1
BAK1021D5	RD								12.0	1
BAK1082E10	RD								14.4	2
ALA VL26G	O								14.4	1
BAK1020C8	RD								15.0	1
BAK1022G9	RD								17.0	1
BAK1053G4	RD								17.0	1
BAK049SG5	PD								18.0	1
BAK1040E5	RD								18.0	1

Table 1 (cont'd)

Kabat numbering	Mean TF1 (nM)	TF1 exp
BAK27806	44	
BAK502G9	9	
BAK1063F1	20.0	1
BAK0494B6	21.8	4
BAK1010C5	22.0	1
BAK0498H4	22.0	3
BAK0501B8	22.3	3
BAK1049D7	23.0	2
BAK0531E2	23.0	1
BAK0433C4	23.1	3
BAK1008E9	23.3	4
BAK0469C8	27.0	1
BAK0442E6	28.2	5
BAK1047E3	29.3	3
BAK0472D7	30.0	1
BAK1008A7	31.0	4
BAK1083H4	32.0	1
BAK0495A4	34.0	1
BAK0782D5	34.6	5
BAK0502C3	38.0	1
BAK1007H6	39.3	3
BAK1004E6	40.5	2
BAK1049G1	43.0	1
BAK0484B2	46.0	1
BAK0502D5	28.4	6
	28.3	4

Phage Display — PD
Ribosome Display — RD
Point mutations — O
Germlining — GL

101

Table 2

Binding specificity of anti-human IL-13 antibodies

	Human IL-13	Human IL-13 variant	Non-human primate IL-13
BAK278D6	+	+	+
BAK502G9	+	+	+
BAK615E3	+	−	−

5

Table 3a

Kinetic analysis of anti-human IL-13 antibodies

IgG	Off-rate (s^{-1})	On-rate $(M^{-1} s^{-1})$	KD (pM)
BAK278D6	$7.41e^{-3}$	$5.49e^{5}$	13500
BAK502G9	$4.09e^{-4}$	$2.49e^{6}$	178
BAK1167F2	$4.05e^{-4}$	$2.99e^{6}$	136
BAK1183H4	$3.00e^{-4}$	$3.7e^{6}$	81

10

Table 3b

Kinetic analysis of anti-murine IL-13 antibodies

IgG	Off-rate (s^{-1})	On-rate $(M^{-1} s^{-1})$	KD (pM)
BAK209B11	$1.98e^{-2}$	$3.9e^{6}$	5100

15

Table 4

Pharmacokinetics of BAK502G9 in 4 allergic but non-challenged
cynomolgus primates (2 male/2 female) after a single 10mg/kg
5   *i.v bolus dose over 29 days. BAK502G9 levels in serum were*
measured by ELISA (mean data).

C_{max} (t=0.25h) (μg/mL)	349.04	
Vd_{inf} (mL.kg^{-1})	75.03	<80mL/kg, infers no tissue binding.
Cl_{inf} (mL.hr^{-1}.kg^{-1})	0.23	. .
AUC_{inf} (mg.h.mL^{-1})	42.99	
AUC_{ext} (%)	17.34	<30% so clearance and vol. of distribution should be accurate.
$T_{0.5}$ (h)	223.55	

$\underline{Vd}_{inf}$ = volume of distribution over time 0 - infinity,
10   calculated from the extrapolated AUC.

$\underline{Cl}_{inf}$ = clearance over time 0 - infinity, calculated from the
extrapolated AUC.

$\underline{AUC}_{inf}$ = area under the curve (measure of total drug exposure)
over time 0 - infinity, including an extrapolated term based
15   on the elimination rate constant (k) and the last observed
serum drug concentration.

$\underline{AUC}_{ext}$ = percentage of the total AUC that is extrapolated.

$\underline{T}_{0.5}$ = Drug half-life in the terminal elimination phase.

ADDITIONAL STATEMENTS OF INVENTION:

1.   An isolated specific binding member for human IL-13, comprising an antibody antigen-binding site which is composed
5   of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the set of CDR's consists of a set of CDR's selected
10   from the group consisting of:
     the BAK278D6 set of CDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid
15   sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6,
     a set of CDR's which contains one or two amino acid substitutions compared with the BAK278D6 set of CDR's, and
20   each set of CDR's as shown for individual clones in Table 1.

2.   An isolated specific binding member according to statement 1 wherein the one or two substitutions are at one or
25   two of the following residues within the CDRs, using the standard numbering of Kabat.

31, 32, 34 in HCDR1

30   52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2

96, 97, 98, 99, 101 in HCDR3

26, 27, 28, 30, 31 in LCDR1

56 in LCDR2

95A, 97 in LCDR3

3.   An isolated specific binding member according to
statement 2 wherein the one or two substitutions are made at
the following positions from among the identified groups of
possible substitute residues for each position:

Position of   Substitute Residue
substitution   selected from the group
                       consisting of

31 in HCDR1:   Q, D, L, G and E
32 in HCDR1:   T
34 in HCDR1:   V, I and F

52 in HCDR2:   D, N, A, R, G and E
52A in HCDR2:   D, G, T, P, N and Y
53 in HCDR2:   D, L, A, P, T, S, I and R
54 in HCDR2:   S, T, D, G, K and I
56 in HCDR2:   T, E, Q, L, Y, N, V, A, M and G
58 in HCDR2:   I, L, Q, S, M, H, D and K
60 in HCDR2:   R
61 in HCDR2:   R
62 in HCDR2:   K and G
64 in HCDR2:   R
65 in HCDR2:   K

96 in HCDR3:   R and D
97 in HCDR3:   N, D, T and P
98 in HCDR3:   R

99 in HCDR3:   S, A, I, R, P and K

101 in HCDR3:   Y

26 in LCDR1:   D and S

5   27 in LCDR1:   I, L, M, C, V, K, Y, F, R, T, S, A, H and G

28 in LCDR1:   V

30 in LCDR1:   G

31 in LCDR1:   R

10   56 in LCDR2:   T

95A in LCDR3:   N

97 in LCDR3:   I.

15.   4.   An isolated specific binding member according to statement 3 wherein there are two substitutions compared with the BAK278D6 set of CDR's, at HCDR3 residue 99 and LCDR1 residue 27.

20   5.   An isolated specific binding member according to statement 4 comprising the BAK278D6 set of CDR's with a substitution at HCDR3 residue 99 selected from the group consisting of S, A, I, R, P and K, and/or a substitution at LCDR1 residue 27 selected from the group consisting of I, L, 25 M, C, V, K, Y, F, R, T, S, A, H and G.

6.   An isolated specific binding member according to statement 4 comprising the BAK278D6 set of CDR's with S substituted for N at HCDR3 residue 99 and/or I substituted for 30 N at LCDR 1 residue 27.

7.   An isolated specific binding member according to any one of statements 1 to 6 wherein HCDR1, HCDR2 and HCDR3 of the VH

domain are within a germ-line framework and/or LCDR1, LCDR2 and LCDR3 of the VL domain are within a germ-line framework.

8. An isolated specific binding member according to statement 7 wherein the HCDR1, HCDR2 and HCDR3 of the VH domain are within germ-line framework VH1 DP14.

9. An isolated specific binding member according to statement 7 or statement 8 wherein the HCDR1, HCDR2 and HCDR3 of the VH domain are within germ-line framework VL Vλ3 3h.

10. An isolated specific binding member according to any one of statements 1 to 9 which binds a human IL-13 variant in which arginine at position 130 is replaced by glutamine.

11. An isolated specific binding member according to any one of statements 1 to 10 which binds non-human primate IL-13.

12. An isolated specific binding member according to statement 11 wherein the non-human primate IL-13 is rhesus or cynomolgus.

13. A specific binding member according to any one of statements 8 to 12 comprising the BAK502G9 VH domain (SEQ ID NO: 15).

14. A specific binding member according to any one of statements 8 to 13 comprising the BAK502G9 VL domain (SEQ ID NO: 16).

15. A specific binding member according to any one of statements 1 to 14 that binds IL-13 with affinity equal to or better than the affinity of an IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the

BAK502G9 VL domain (SEQ ID NO: 16), the affinity of the specific binding member and the affinity of the antigen-binding site being as determined under the same conditions.

5   16.   A specific binding member according to any one of statements 1 to 15 that neutralizes human IL-13.

17. A specific binding member according to statement 16 that neutralizes human IL-13, with a potency equal to or better

10   than the potency of a IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain (SEQ ID NO: 16), the potency of the specific binding member and the potency of the antigen-binding site being as determined under the same conditions.

15

18.   A specific binding member according to any one of statements 1 to 17 that comprises an scFv antibody molecule.

19.   A specific binding member according to any one of

20   statements 1 to 17 that comprises an antibody constant region.

20.   A specific binding member according to statement 19 that comprises a whole antibody.

25   21.   A specific binding member according to statement 20 wherein the whole antibody is IgG4.

22.   An isolated antibody VH domain of a specific binding member according to any one of statements 1 to 21.

30

23.   An isolated antibody VL domain of a specific binding member according to any one of statements 1 to 21.

24. A composition comprising a specific binding member, antibody VH domain or antibody VL according to any one of statements 1 to 23 and at least one additional component.

5   25. A composition according to statement 24 comprising a pharmaceutically acceptable excipient, vehicle or carrier.

26. An isolated nucleic acid which comprises a nucleotide sequence encoding a specific binding member or antibody VH or

10   VL domain of a specific binding member according to any one of statements 1 to 23.

27. A host cell *in vitro* transformed with nucleic acid according to statement 26.

15

28. A method of producing a specific binding member or antibody VH or VL domain, the method comprising culturing host cells according to statement 27 under conditions for production of said specific binding member or antibody VH or

20   VL domain.

29. A method according to statement 28 further comprising isolating and/or purifying said specific binding member or antibody VH or VL variable domain.

25

30. A method according to statement 28 or statement 29 further comprising formulating the specific binding member or antibody VH or VL variable domain into a composition including at least one additional component.

30

31. A method for producing an antibody antigen-binding domain specific for human IL-13, the method comprising

    providing, by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid

sequence of a parent VH domain comprising HCDR 1, HCDR2 and HCDR3, wherein the parent VH domain HCDR1, HCDR2 and HCDR3 are the BAK278D6 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino

5 acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, or the BAK502G9 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 7, the HCDR2 has the amino acid sequence of SEQ ID NO: 8, the HCDR3 has the amino acid sequence of SEQ ID NO: 9, a VH

10 domain which is an amino acid sequence variant of the parent VH domain, and optionally combining the VH domain thus provided with one or more VL domains to provide one or more VH/VL combinations; and

testing said VH domain which is an amino acid sequence

15 variant of the parent VH domain or the VH/VL combination or combinations to identify an antibody antigen binding domain specific for human IL-13.

32. A method according to statement 31 wherein the parent VH

20 domain amino acid sequence is selected from the group consisting of SEQ ID NO: 13 and SEQ ID NO: 15.

33. A method according to statement 31 or statement 32 wherein said one or more VL domains is provided by way of

25 addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VL domain comprising LCDR 1, LCDR2 and LCDR3, wherein the parent VL domain LCDR1, LCDR2 and LCDR3 are the BAK278D6 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ

30 ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, the LCDR3 has the amino acid sequence of SEQ ID NO: 6, or the BAK502G9 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 10, the LCDR2 has the amino acid sequence of SEQ ID NO: 11, the LCDR3 has the amino acid

sequence of SEQ ID NO: 12, producing one or more VL domains
each of which is an amino acid sequence variant of the parent
VL domain.

5 34. A method according to statement 33 wherein the parent VL
domain amino acid sequence is selected from the group
consisting of SEQ ID NO: 14 and SEQ ID NO: 16.

35. A method for producing an antibody antigen-binding domain
10 specific for human IL-13, the method comprising
      providing, by way of addition, deletion, substitution or
insertion of one or more amino acids in the amino acid
sequence of a parent VH domain comprising HCDR 1, HCDR2 and
HCDR3, wherein the parent VH domain HCDR1, HCDR2 and HCDR3 are
15 the BAK167A11 set of HCDR's, defined wherein the HCDR1 has the
amino acid sequence of SEQ ID NO: 55, the HCDR2 has the amino
acid sequence of SEQ ID NO: 56, the HCDR3 has the amino acid
sequence of SEQ ID NO: 57, the BAK615E3 set of HCDR's, defined
wherein the HCDR1 has the amino acid sequence of SEQ ID NO:
20 153, the HCDR2 has the amino acid sequence of SEQ ID NO: 154,
the HCDR3 has the amino acid sequence of SEQ ID NO: 155, the
BAK582F7 set of HCDR's, defined wherein the HCDR1 has the
amino acid sequence of SEQ ID NO: 141, the HCDR2 has the amino
acid sequence of SEQ ID NO: 142, the HCDR3 has the amino acid
25 sequence of SEQ ID NO: 143, or the BAK612B5 set of HCDR's,
defined wherein the HCDR1 has the amino acid sequence of SEQ
ID NO: 147, the HCDR2 has the amino acid sequence of SEQ ID
NO: 148, the HCDR3 has the amino acid sequence of SEQ ID NO:
149, a VH domain which is an amino acid sequence variant of
30 the parent VH domain, and optionally combining the VH domain
thus provided with one or more VL domains to provide one or
more VH/VL combinations; and
      testing said VH domain which is an amino acid sequence
variant of the parent VH domain or the VH/VL combination or

combinations to identify an antibody antigen binding domain specific for human IL-13.

36. A method according to statement 35 wherein the parent VH domain amino acid sequence is selected from the group consisting of SEQ ID NO: 55 and SEQ ID NO: 33.

37. A method according to statement 35 or statement 36 wherein said one or more VL domains is provided by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VL domain comprising LCDR 1, LCDR2 and LCDR3, wherein the parent VL domain LCDR1, LCDR2 and LCDR3 are the BAK167A11 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 58, the LCDR2 has the amino acid sequence of SEQ ID NO: 59, the LCDR3 has the amino acid sequence of SEQ ID NO: 60, the BAK615E3 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 156, the LCDR2 has the amino acid sequence of SEQ ID NO: 157, the LCDR3 has the amino acid sequence of SEQ ID NO: 158, the BAK582F7 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 144, the LCDR2 has the amino acid sequence of SEQ ID NO: 145, the LCDR3 has the amino acid sequence of SEQ ID NO: 146, or the BAK612B5 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 150, the LCDR2 has the amino acid sequence of SEQ ID NO: 151, the LCDR3 has the amino acid sequence of SEQ ID NO: 152, producing one or more VL domains each of which is an amino acid sequence variant of the parent VL domain.

38. A method according to statement 37 wherein the parent VL domain amino acid sequence is selected from the group consisting of SEQ ID NO: 24 and SEQ ID NO: 34.

39. A method according to any one of statements 31 to 34 wherein said VH domain which is an amino acid sequence variant of the parent VH domain is provided by CDR mutagenesis.

5 40. A method according to any one of statements 35 to 38 wherein said VH domain which is an amino acid sequence variant of the parent VH domain is provided by CDR mutagenesis.

41. A method according to any one of statements 31 to 40
10 further comprising providing the antibody antigen binding site within an IgG, scFv or Fab antibody molecule.

42. A method of producing a specific binding member that binds human IL-13, which method comprises:
15   providing starting nucleic acid encoding a VH domain or a starting repertoire of nucleic acids each encoding a VH domain, wherein the VH domain or VH domains either comprise a HCDR1, HCDR2 and/or HCDR3 to be replaced or lack a HCDR1, HCDR2 and/or HCDR3 encoding region;
20   combining said starting nucleic acid or starting repertoire with donor nucleic acid or donor nucleic acids encoding or produced by mutation of the amino acid sequence of the HCDR1 (SEQ ID NO: 1) or HCDR1 (SEQ ID NO: 7), HCDR2 (SEQ ID NO: 2) or HCDR2 (SEQ ID NO: 8) and/or HCDR3 (SEQ ID NO: 3)
25 or HCDR3 (SEQ ID NO: 9) such that said donor nucleic acid is or donor nucleic acids are inserted into the CDR1, CDR2 and/or CDR3 region in the starting nucleic acid or starting repertoire, so as to provide a product repertoire of nucleic acids encoding VH domains;
30   expressing the nucleic acids of said product repertoire to produce product VH domains;
        optionally combining said product VH domains with one or more VL domains;

selecting a specific binding member specific for human IL-13, which specific binding member comprises a product VH domain and optionally a VL domain; and

recovering said specific binding member or nucleic acid
5   encoding it.

43.   A method according to statement 42 wherein the donor nucleic acids are produced by mutation of said HCDR1 and/or HCDR2.

10

44.   A method according to statement 42 wherein the donor nucleic acid is produced by mutation of HCDR3.

45.   A method according to statement 44 comprising
15   providing the donor nucleic acid by mutation of nucleic acid encoding the amino acid sequence of HCDR3 (SEQ ID NO: 3) or HCDR3 (SEQ ID NO: 9).

46.   A method according to statement 42 comprising providing
20   the donor nucleic acid by random mutation of nucleic acid.

47.   A method according to any one of statements 42 to 46 further comprising attaching a product VH domain that is comprised within the recovered specific binding member to an
25   antibody constant region.

48.   A method according to any one of statements 42 to 46 comprising providing an IgG, scFv or Fab antibody molecule comprising the product VH domain and a VL domain.

30

49.   A method according to any one of statements 31 to 48, further comprising testing the antibody antigen-binding domain or specific binding member that binds human IL-13 for ability to neutralize human IL-13.

50. A method according to statement 49 wherein a specific binding member that comprises an antibody fragment that binds and neutralizes human IL-13 is obtained.

5

51. A method according to statement 50 wherein the antibody fragment is an scFv antibody molecule.

52. A method according to statement 50 wherein the antibody

10 fragment is an Fab antibody molecule.

53. A method according to statement 51 or statement 52 further comprising providing the VH domain and/or the VL domain of the antibody fragment in a whole antibody.

15

54. A method according to any one of statements 31 to 53 further comprising formulating the specific binding member that binds IL-13, antibody antigen-binding site or an antibody VH or VL variable domain of the specific binding member or

20 antibody antigen-binding site that binds IL-13, into a composition including at least one additional component.

55. A method according to any one of statements 31 to 54 further comprising binding a specific binding member that

25 binds human IL-13 to IL-13 or a fragment of IL-13.

56. A method comprising binding a specific binding member that binds IL-13 according to any one of statements 1 to 21 to human IL-13 or a fragment of human IL-13.

30

57. A method according to statement 55 or statement 56 wherein said binding takes place *in vitro*.

58. A method according to any one of statements 55 to 57 comprising determining the amount of binding of specific binding member to IL-13 or a fragment of IL-13.

5   59. A method according to any one of statements 31 to 58 further comprising use of the specific binding member in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel
10   disease and Hodgkin's lymphoma.

60. Use of a specific binding member according to any one of statements 1 to 21 in the manufacture of a medicament for treatment of a disease or disorder selected from the group
15   consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

61.. A method of treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis,
20   allergic rhinitis, fibrosis and Hodgkin's lymphoma, the method comprising administering a specific binding member according to any one of statements 1 to 21 to a patient with the disease or disorder or at risk of developing the disease or disorder.

25   62. An isolated specific binding member for human IL-13, comprising an antibody antigen-binding domain site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises
30   HCDR1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein

HCDR1 is of amino acid sequence which has the formula
HX_1 HX_2 G HX_3 S

wherein

HX$_1$ is selected from the group consisting of N, Q, D, L, G and E,

HX$_2$ is selected from the group consisting of Y and T,

5   HX$_3$ is selected from the group consisting of V, I, F and L,

HCDR2 is of amino acid sequence which has the formula

10   W I HX$_4$ HX$_5$ HX$_6$ HX$_7$ G HX$_8$ T HX$_9$ Y HX$_{10}$ HX$_{11}$ HX$_{12}$ F HX$_{13}$ HX$_{14}$

wherein

HX$_4$ is selected from the group consisting of S, D, N, A, R, G and E,

HX$_5$ is selected from the group consisting of A, D, G, T,

15   P, N and Y,

HX$_6$ is selected from the group consisting of N, D, L, A, P, T, S, I and R,

HX$_7$ is selected from the group consisting of N, S, T, D, G, K and I,

20   HX$_8$ is selected from the group consisting of D, T, E, Q, L, Y, N, V, A, M and G,

HX$_9$ is selected from the group consisting of N, I, L, Q, S, M, H, D and K,

HX$_{10}$ is selected from the group consisting of G and R,

25   HX$_{11}$ is selected from the group consisting of Q and R,

HX$_{12}$ is selected from the group consisting of E, K and G,

HX$_{13}$ is selected from the group consisting of Q and R,

HX$_{14}$ is selected from the group consisting of G and K,

30   HCDR3 is of amino acid sequence which has the formula

D HX$_{15}$ HX$_{16}$ HX$_{17}$ HX$_{18}$ W A R W HX$_{19}$ F HX$_{20}$ L

wherein

HX_{15} is selected from the group consisting of S, R and D,

HX_{16} is selected from the group consisting of S, N, D, T and P,

HX_{17} is selected from the group consisting of S and R,

HX_{18} is selected from the group consisting of S, N, A, I, R, P and K,

HX_{19} is selected from the group consisting of F and Y,

HX_{20} is selected from the group consisting of D and Y,

LCDR1 is of amino acid sequence which has the formula

G G LX_1 LX_2 LX_3 G LX_4 LX_5 L V H

wherein

LX_1 is selected from the group consisting of N, D and S,

LX_2 is selected from the group consisting of N, I, L, M, C, V, K, Y, F, R, T, S, A, H and G,

LX_3 is selected from the group consisting of I and V,

LX_4 is selected from the group consisting of S and G,

LX_5 is selected from the group consisting of K and R,

LCDR2 is of amino acid sequence which has the formula

D D G D R P LX_6

wherein

LX_6 is selected from the group consisting of S and T,

LCDR3 is of amino acid sequence which has the formula

Q V W D T G S LX_7 P V LX_8

wherein

LX₇ is selected from the group consisting of D and N,

LX₈ is selected from the group consisting of V and I.

63. An isolated specific binding member according to statement 62, wherein

HX₁ is selected from the group consisting of D and N,

HX₂ is Y,

HX₃ is L,

HX₄ is selected from the group consisting of S and G,

HX₅ is selected from the group consisting of T and A,

HX₆ is N,

HX₇ is selected from the group consisting of N and I,

HX₈ is D,

HX₉ is selected from the group consisting of N, D and K,

HX₁₀ is G,

HX₁₂ is selected from the group consisting of E and G,

HX₁₃ is Q,

HX₁₉ is F,

LX₁ is selected from the group consisting of N and S,

LX₂ is selected from the group consisting of N, Y, T, S, and I,

LX₆ is S,

LX₇ is D.

64. An isolated specific binding member according to statement 62, wherein

HX₁ is selected from the group consisting of N and D,

HX₂ is Y,

HX₃ is L,

HX₄ is selected from the group consisting of S and G,

HX₅ is selected from the group consisting of A and T,

HX$_6$ is N,

HX$_7$ is N,

HX$_8$ is selected from the group consisting of D and G,

HX$_9$ is selected from the group consisting of I, S, N and

5 D,

HX$_{11}$ is Q,

HX$_{12}$ is E and K,

HX$_{14}$ is G,

HX$_{15}$ is S,

10 HX$_{16}$ is selected from the group consisting of S and N,

HX$_{17}$ is S,

HX$_{18}$ is selected from the group consisting of S and N,

HX$_{19}$ is F,

HX$_{20}$ is D,

15

LX$_1$ is selected from the group consisting of N and D,

LX$_3$ is I,

LX$_8$ is V.

20 65. An isolated specific binding member according to
statement 62, wherein

HX$_7$ is selected from the group consisting of N, S, T, D,
G and K,

25 HX$_8$ is selected from the group consisting of D, T, E, Q,
L, Y, N, V, A, M,

HX$_9$ is selected from the group consisting of N, I, L, Q,
S, M and H,

HX$_{10}$ is G,

30 HX$_{11}$ is Q,

HX$_{12}$ is F,

HX$_{13}$ is Q,

HX$_{14}$ is G,

HX$_{15}$ is S,

HX_{16} is selected from the group consisting of N and S,

HX_{17} is S,

HX_{18} is selected from the group consisting of N and S,

HX_{19} is F,

5   HX_{20} is D,

LX_1 is N,

LX_2 is selected from the group consisting of N and I,

LX_3 is I,

10   LX_4 is S,

LX_5 is K,

LX_6 is S,

LX_7 is D,

LX_8 is V.

15

66.   An isolated specific binding member according to statement 65, wherein

HX_1 is selected from the group consisting of N, Q and D,

20   HX_3 is selected from the group consisting of L, V and I,

HX_4 is selected from the group consisting of S, N, A and R,

HX_5 is selected from the group consisting of A, D, T, G, N and Y,

25   HX_6 is selected from the group consisting of N, A, P, S, D and I,

HX_7 is selected from the group consisting of N, T, D and G,

HX_8 is selected from the group consisting of D, Q, Y and

30   N,

HX_9 is selected from the group consisting of N, Q, S and I.

67. A specific binding member according to any one of statements 62 to 66 that neutralizes human IL-13.

68. A specific binding member according to statement 67 that
5 neutralizes human IL-13, with a potency equal to or better than the potency of a IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain (SEQ ID NO: 16), the potency of the specific binding member and the potency of the antigen-binding site being as
10 determined under the same conditions.

69. A specific binding member according to any one of statements 62 to 68 that comprises an scFv antibody molecule.

15 70. A specific binding member according to any one of statements 62 to 68 that comprises an antibody constant region.

71. A specific binding member according to statement 70 that
20 comprises a whole antibody.

72. A specific binding member according to statement 71 wherein the whole antibody is IgG4.

25 73. An isolated specific binding member according to any one of statements 62 to 72 which binds a human IL-13 variant in which arginine at position 130 is replaced by glutamine.

74. An isolated specific binding member according to any one
30 of statements 62 to 72 which binds non-human primate IL-13.

75. An isolated specific binding member according to statement 74 wherein the non-human primate IL-13 is rhesus or cynomolgus.

76. An isolated antibody VH domain of a specific binding member according to any one of statements 62 to 75.

5 77. An isolated antibody VL domain of a specific binding member according to any one of statements 62 to 75.

78. A composition comprising the specific binding member, antibody VH domain or antibody VL domain of any one of
10 statements 62 to 77 and at least one additional component.

79. A composition according to statement 78 comprising a pharmaceutically acceptable excipient, vehicle or carrier.

15 80. An isolated nucleic acid which comprises a nucleotide sequence encoding a specific binding member or antibody VH or VL domain of a specific binding member according to any one of statements 62 to 77.

20 81. A host cell *in vitro* transformed with nucleic acid according to statement 80.

82. A method of producing a specific binding member. or antibody VH or VL domain, the method comprising culturing host
25 cells according to statement 81 under conditions for production of said specific binding member or antibody VH or VL domain.

83. A method according to statement 82 further comprising
30 isolating and/or purifying said specific binding member or antibody VH or VL variable domain.

84. A method according to statement 82 or statement 83 further comprising formulating the specific binding member or

antibody VH or VL variable domain into a composition including at least one additional component.

85.   A method according to any one of statements 82 to 84 further comprising binding a specific binding member that binds human IL-13 to IL-13 or a fragment of IL-13.

86.   A method comprising binding a specific binding member that binds IL-13 according to any one of statements 62 to 75 to human IL-13 or a fragment of human IL-13.

87.   A method according to statement 85 or statement 86 wherein said binding takes place *in vitro*.

88.   A method according to any one of statements 85 to 87 comprising determining the amount of binding of specific binding member to IL-13 or a fragment of IL-13.

89.   A method according to any one of statements 82 to 84 further comprising use of the specific binding member in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

90.   Use of a specific binding member according to any one of statements 62 to 75 in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

91.   A method of treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis and Hodgkin's lymphoma, the method

comprising administering a specific binding member according to any one of statements 62 to 75 to a patient with the disease or disorder or at risk of developing the disease or disorder.

5

BAK278D6

HEAVY CHAIN

CDR1- SEQ ID NO 1:   NYGLS
5   CDR2- SEQ ID NO 2:   WISANGDTNYGQEFQG
CDR3- SEQ ID NO 3:   DSSSNWARWFFDL

BAK278D6

10   LIGHT CHAIN

CDR1- SEQ ID NO 4:   GGNNIGSKLVH
CDR2- SEQ ID NO 5:   DDGDRPS
CDR3- SEQ ID NO 6:   QVWDTGSDPVV

15

BAK502G9

HEAVY CHAIN

20   CDR1-SEQ ID NO 7:   NYGLS
CDR2-SEQ ID NO 8:   WISANGDTNYGQEFQG
CDR3-SEQ ID NO 9:   DSSSSWARWFFDL

LIGHT CHAIN

25

CDR1-SEQ ID NO 10:   GGNIIGSKLVH
CDR2-SEQ ID NO 11:   DDGDRPS
CDR3-SEQ ID NO 12:   QVWDTGSDPVV

30

BAK278D6

HEAVY CHAIN DOMAIN

SEQ ID NO 13:

EVQLVQSGAEVKKPGASVKVSCKASGYTFRNYGLSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRITMTTETSTNTAHMELRSLRSDDTAVYYCVRDSSSNWARWFFDLWGKGTMVTVSS

BAK278D6

5   LIGHT CHAIN DOMAIN

SEQ ID NO 14:
SYVLTQPPSVSVAPGQTARIPCGGNNIGSKLVHWYQQKPGQAPVLVVYDDGDRPSGIPERFS
GSNSGNTATLTISRIDAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

10

BAK502G9

15 HEAVY CHAIN DOMAIN

SEQ ID NO 15:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

20

BAK502G9

LIGHT CHAIN DOMAIN

SEQ ID NO 16:
25 SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK278D6

30 HEAVY CHAIN

FR1- SEQ ID NO 17:   EVQLVQSGAEVKKPGASVKVSCKASGYTFR
FR2- SEQ ID NO 18:   WVRQAPGQGLEWMG
FR3- SEQ ID NO 19:   RITMTTETSTNTAHMELRSLRSDDTAVYYCVR

BAK278D6

LIGHT CHAIN

5 FR1- SEQ ID NO 20: SYVLTQPPSVSVAPGQTARIPC

FR2- SEQ ID NO 21: WYQQKPGQAPVLVVY

FR3- SEQ ID NO 22: GIPERFSGSNSGNTATLTISRIDAGDEADYYC

BAK167A11

10 HEAVY CHAIN DOMAIN

SEQ ID NO 23:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD

SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVGAAGEGYYGYWGRGTLVTVSS

15

BAK167A11

LIGHT CHAIN DOMAIN

SEQ ID NO 24:

20 NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYDDNQRPSGVPDR

FSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSNNDVFGGGTKVTVL

BAK209B11

HEAVY CHAIN DOMAIN

25

SEQ ID NO 25:

QVQLQESGGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVSSISASGDSTFYA

DSVKGRFTISRDNNKNMVFLQVNSLRADDTAVYFCAKDWSQWLVGDAFDVWGRGTTVTVSS

30 *BAK209B11*

LIGHT CHAIN DOMAIN

SEQ ID NO 26:

DIQLTQSPSTLSASVGDRVTITCRASQSVSLWVAWYQQRPGKAPKLLIYDGSTLQSGVPARF
SGSGSGTEFTLTISSLQPDDFATYYCQQYKTFSTFGQGTKVEIKRA

BAK502G9

HEAVY CHAIN

FR1- SEQ ID NO 27:   QVQLVQSGAEVKKPGASVKVSCKASGYTFT
FR2- SEQ ID NO 28:   WYRQAPGQGLEWMG
FR3- SEQ ID NO 29:   RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR

BAK502G9

LIGHT CHAIN

FR1- SEQ ID NO 30:   SYVLTQPPSVSVAPGKTARITC
FR2- SEQ ID NO 31:   WYQQKPGQAPVLVIY
FR3- SEQ ID NO 32:   GIPERFSGSNSGNTATLTISRVEAGDEADYYC

BAK615E3

HEAVY CHAIN DOMAIN

SEQ ID NO 33:
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVGKATTEEGYYGYWGRGTLVTVSS

BAK615E3

LIGHT CHAIN DOMAIN

SEQ ID NO 34:
NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYDDNQRPSGVPDR
FSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSNNDVFGGGTKVTVL

5   *BAK1167F2*

HEAVY CHAIN DOMAIN

SEQ ID NO 35:

10 QVQLVQSGAEVKKPGASVKVSCKASGYTFEQTGVSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1167F2
LIGHT CHAIN DOMAIN

15

SEQ ID NO 36:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

20

BAK1183H4
HEAVY CHAIN DOMAIN

SEQ ID NO 37:

.25 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWINYDGGNTQYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1183H4
LIGHT CHAIN DOMAIN

30

SEQ ID NO 38:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1105H3

HEAVY CHAIN DOMAIN

SEQ ID NO 39:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISGLNGETLYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1105H3
LIGHT CHAIN DOMAIN

SEQ ID NO 40:

SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1111D10
HEAVY CHAIN DOMAIN

SEQ ID NO 41:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWIATPDGQTSYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSNSSWARWFFDLWGRGTLVTVSS

BAK1111D10

LIGHT CHAIN DOMAIN

SEQ ID NO 42:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1167F4

HEAVY CHAIN DOMAIN

SEQ ID NO 43:

5. QVQLVQSGAEVKKPGASVKVSCKASGYTFIDTGVSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1167F4

LIGHT CHAIN DOMAIN

10

SEQ ID NO 44:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

15

<u>BAK1184C8</u>

HEAVY CHAIN DOMAIN

SEQ ID NO 45:

20 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISGSNGYTSYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1184C8

LIGHT CHAIN DOMAIN

25

SEQ ID NO 46:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

30

BAK1185E1

HEAVY CHAIN DOMAIN

SEQ ID NO 47:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWINDATGDTQYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS .

BAK1185E1 .

5 LIGHT CHAIN DOMAIN

SEQ ID NO 48:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

10

BAK1185F8
HEAVY CHAIN DOMAIN

15 SEQ ID NO 49:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGLSWVRQAPGQGLEWMGWIRNIDGYTIYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1185F8

20 LIGHT CHAIN DOMAIN

SEQ ID NO 50:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

25

BAK1187B4
HEAVY CHAIN DOMAIN

SEQ ID NO 51:
30 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWIDDDSGTTIYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1187B4
LIGHT CHAIN DOMAIN

SEQ ID NO 52:

SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1166G2
HEAVY CHAIN DOMAIN

SEQ ID NO 53:

QVQLVQSGAEVKKPGASVKVSCKASGYTFANTGISWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

BAK1166G2
LIGHT CHAIN DOMAIN

SEQ ID NO 54:

SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK167A11
HEAVY CHAIN

CDR1- SEQ ID NO 55: SYAMS
CDR2- SEQ ID NO 56: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 57: VGAAGEGYYGY

BAK167A11
LIGHT CHAIN

CDR1- SEQ ID NO 58: TRSSGSIASNYVQ
CDR2- SEQ ID NO 59: DDNQRPS
CDR3- SEQ ID NO 60: QSYDSNNDV

BAK1167F2
HEAVY CHAIN

5   CDR1- SEQ ID NO 61: QTGVS

CDR2- SEQ ID NO 62: WISANGDTNYGQEFQG

CDR3- SEQ ID NO 63: DSSSSWARWFFDL

10

BAK1167F2
LIGHT CHAIN

15   CDR1- SEQ ID NO 64: GGNIIGSKLVH

CDR2- SEQ ID NO 65: DDGDRPS

CDR3- SEQ ID NO 66: QVWDTGSDPVV

BAK1166G2

20   HEAVY CHAIN

CDR1- SEQ ID NO 67: NTGIS

CDR2- SEQ ID NO 68: WISANGDTNYGQEFQG

CDR3- SEQ ID NO 69: DSSSSWARWFFDL

25

BAK1166G2
LIGHT CHAIN

CDR1- SEQ ID NO 70: GGNIIGSKLVH

30   CDR2- SEQ ID NO 71: DDGDRPS

CDR3- SEQ ID NO 72: QVWDTGSDPVV

BAK1184C8
HEAVY CHAIN

CDR1- SEQ ID NO 73: NYGLS

CDR2- SEQ ID NO 74: WISGNGYTSYGKEFQG

CDR3- SEQ ID NO 75: DSSSSWARWFFDL

5

BAK1184C8

LIGHT CHAIN

CDR1- SEQ ID NO 76: GGNIIGSKLVH

10   CDR2- SEQ ID NO 77: DDGDRPS

CDR3- SEQ ID NO 78: QVWDTGSDPVV

BAK1185E1

HEAVY CHAIN

15

CDR1- SEQ ID NO 79: NYGLS

CDR2- SEQ ID NO 80: WINDTGDTQYGQEFQG

CDR3- SEQ ID NO 81: DSSSSWARWFFDL

20   *BAK1185E1*

LIGHT CHAIN

CDR1- SEQ ID NO 82: GGNIIGSKLVH

CDR2- SEQ ID NO 83: DDGDRPS

25   CDR3- SEQ ID NO 84: QVWDTGSDPVV

BAK1167F4

HEAVY CHAIN

30   CDR1- SEQ ID NO 85: DTGVS

CDR2- SEQ ID NO 86: WISANGDTNYGQEFQG

CDR3- SEQ ID NO 87: DSSSSWARWFFDL

BAK1167F4

LIGHT CHAIN

CDR1- SEQ ID NO 88: GGNIIGSKLVH
5 CDR2- SEQ ID NO 89: DDGDRPS
CDR3- SEQ ID NO 90: QVWDTGSDPVV

BAK1111D10

HEAVY CHAIN

10

CDR1- SEQ ID NO 91: NYGLS
CDR2- SEQ ID NO 92: WIATDGQTSYGQEFQG
CDR3- SEQ ID NO 93: DSSSSWARWFFDL

15 *BAK1111D10*

LIGHT CHAIN

CDR1- SEQ ID NO 94: GGNIIGSKLVH
CDR2- SEQ ID NO 95: DDGDRPS
20 CDR3- SEQ ID NO 96: QVWDTGSDPVV

BAK1183H4

HEAVY CHAIN

25 CDR1- SEQ ID NO 97: NYGLS
CDR2- SEQ ID NO 98: WINYGGNTQYGQEFQG
CDR3- SEQ ID NO 99: DSSSSWARWFFDL

BAK1183H4

30 LIGHT CHAIN

CDR1- SEQ ID NO 100: GGNIIGSKLVH
CDR2- SEQ ID NO 101: DDGDRPS
CDR3- SEQ ID NO 102: QVWDTGSDPVV

BAK1185H8

HEAVY CHAIN

5   CDR1- SEQ ID NO 103: DYGLS

     CDR2- SEQ ID NO 104: WRINDGYTIYGQEFQG

     CDR3- SEQ ID NO 105: DSSSSWARWFFDL

     BAK1185H8

10   LIGHT CHAIN

     CDR1- SEQ ID NO 106: GGNIIGSKLVH

     CDR2- SEQ ID NO 107: DDGDRPS

     CDR3- SEQ ID NO 108: QVWDTGSDPVV

15

     BAK278D6

     HEAVY CHAIN- SEQ ID NO: 109

20   CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC

     CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG

     GACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG

     GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT

     GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAACT

25   GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

     BAK278D6

     LIGHT CHAIN- SEQ ID NO:110

30   TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGTAAGACGGCCAGGATTAC

     CTGTGGGGGAAACAACATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG

     CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT

     GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC

CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

5 *BAK502G9*

HEAVY CHAIN- SEQ ID NO:111

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
10 GACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

15 <u>BAK502G9</u>

LIGHT CHAIN- SEQ ID NO:112

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
20 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

25 <u>BAK1105H03</u>

HEAVY CHAIN- SEQ ID NO: 113

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
30 GACAAGGGCTTGAGTGGATGGGATGGATCTCCGGCTTGAACGGCGAGACATTGTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK1105H03
LIGHT CHAIN- SEQ ID NO: 114

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK1111D10
HEAVY CHAIN- SEQ ID NO:115

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCGCAACCCCAGACGGCCAGACAAGCTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAACAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK1111D10
LIGHT CHAIN- SEQ ID NO:116

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK 1167F2
HEAVY CHAIN- SEQ ID NO: 117

CAAGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTGAGCAGACCGGCGTCTCCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK 1167F2
LIGHT CHAIN- SEQ ID NO:118

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK *1167F04*
HEAVY CHAIN- SEQ ID NO:119

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTATCGACACCGGGGTCTCCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK 1167F04
LIGHT CHAIN- SEQ ID NO:120

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC

CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK 1183H4

HEAVY CHAIN- SEQ ID NO:121

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCAACTACGACGGCGGCAACACACAGTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK 1183H4

LIGHT CHAIN- SEQ ID NO:122

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK1184C8

HEAVY CHAIN- SEQ ID NO:123

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCAGCGGGAGCAACGGCTACACATCTTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACGTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK1184C8

LIGHT CHAIN- SEQ ID NO:124

5

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC

10   CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK1185E1

15   HEAVY CHAIN- SEQ ID NO:125

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCAACGACGCCACCGGCGACACACAGTATGGACAG

20   GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK1185E1

25   LIGHT CHAIN- SEQ ID NO:126

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT

30   GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK1185F8

HEAVY CHAIN- SEQ ID NO:127

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAGATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTAGAGTGGATGGGATGGATCCGCAACATCGACGGCTACACAATTTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK1185F8

LIGHT CHAIN- SEQ ID NO:128

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

BAK1187B4

HEAVY CHAIN- SEQ ID NO:129

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCGACGACGACAGCGGCACGACAATATATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTCACCGTCTCCTCA

BAK1187B4

LIGHT CHAIN- SEQ ID NO:130

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
5   CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

10   *BAK1166G02*
HEAVY CHAIN- SEQ ID NO: 131

CAAGTGCAGTTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTTGCGAACACCGGGATCTCGTGGGTGCGACAGGCCCCTG
15   GACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCCGCTGGTTTTTCGATCTCTGGGGTCGGGGGACACTGGTCACCGTCTCCTCA

20   BAK1166G02
LIGHT CHAIN- SEQ ID NO:132

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
25   CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGGATGAGGC
CGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

30

BAK165E7
HEAVY CHAIN- SEQ ID NO: 133

EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISANNGETNYGQ
EFQGRVTMTTETPTNTAHMELRSLTSDDTAVYYCVRDSSSNWARWYFDLWGQGTLVTVSS

BAK165E7

5 LIGHT CHAIN- SEQ ID NO: 134

SYVLTQPPSVSVAPGQTARIPCGGNNIGSKLVHWYQQKPGQAPVLVVYDDGDRPSGIPERFS
GSNSGNTATLTISRIDAGDEADYYCQVWDTGSDPVVFGGGTKLTVLG

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BAK165E7

HEAVY CHAIN

15

CDR1- SEQ ID NO:135 NYGLS
CDR2- SEQ ID NO:136 WISANNGETNYGQEFQG
CDR3- SEQ ID NO:137 DSSSNWARWYFDL

20 *BAK165E7*

LIGHT CHAIN

CDR1- SEQ ID NO:138 GGNNIGSKLVH
CDR2- SEQ ID NO:139 DDGDRPS
25 CDR3- SEQ ID NO:140 QVWDTGSDPVV

BAK582F7

HEAVY CHAIN

30 CDR1- SEQ ID NO 141: SYAMS
CDR2- SEQ ID NO 142: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 143: VGAAGEGYYGY

BAK582F7

LIGHT CHAIN

CDR1-SEQ ID NO 144: TRSSGSIASNYVE
CDR2-SEQ ID NO 145: DDNQRPS
5   CDR3-SEQ ID NO 146: QSYDSNNDV

BAK612B5
HEAVY CHAIN

10   CDR1- SEQ ID NO 147: SYAMS
CDR2- SEQ ID NO 148: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 149: VGRATTDEGYYGY

BAK612B5
15   LIGHT CHAIN

CDR1- SEQ ID NO 150: TRSSGSIASNYVQ
CDR2- SEQ ID NO 151: DDNQRPS
CDR3- SEQ ID NO 152: QSYDSNNDV

20

BAK615E3
25   HEAVY CHAIN

CDR1- SEQ ID NO 153: SYAMS
CDR2- SEQ ID NO 154: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 155: VGKATTEEGYY

30
BAK615E3
LIGHT CHAIN

CDR1- SEQ ID NO 156: TRSSGSIASNYVQ

CDR2- SEQ ID NO 157: DDNQRPS

CDR3- SEQ ID NO 158: QSYDSNNDV

BAK0278D6

HEAVY CHAIN

CDR1- SEQ ID NO 159: AATTATGGTCTCAGC

CDR2- SEQ ID NO 160: TGGATCAGCGCTAATAATGGCGACACAAATTAT

GGACAGGAATTCCAGGGC

CDR3- SEQ ID NO 161: GACTCCAGCAGCAACTGGGCCCGCTGGTTTTTC

GATCTC

BAK278D6

LIGHT CHAIN

CDR1- SEQ ID NO 162: GGGGGAAACAACATTGGAAGTAAACTTGTACAC

CDR2- SEQ ID NO 163: GATGATGGCGACCGGCCCTCA

CDR3- SEQ ID NO 164: CAGGTGTGGGATACTGGTAGTGATCCCGTGGTA

BAK502G9

HEAVY CHAIN

CDR1- SEQ ID NO 165: AATTATGGTCTCAGC

CDR2- SEQ ID NO 166:TGGATCAGCGCTAATAATGGCGACACAAATTATGGACA

GGAATTCCAGGGC

CDR3- SEQ ID NO 167:GACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTC

BAK502G9

LIGHT CHAIN

CDR1- SEQ ID NO 168: GGGGGAAACATCATTGGAAGTAAACTTGTACAC

CDR2- SEQ ID NO 169: GATGATGGCGACCGGCCCTCA

CDR3- SEQ ID NO 170: CAGGTGTGGGATACTGGTAGTGATCCCGTGGTA

CH Domains- SEQ ID NO: 171

5

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL

YSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFP

PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL

TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL

10   VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE

ALHNHYTQKSLSLSLGK

CL Domain- SEQ ID NO: 172

15   QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN

NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

CLAIMS:

1. An isolated specific binding member for human IL-13, comprising an antibody antigen-binding domain site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein

HCDR1 is of amino acid sequence which has the formula

$$HX_1 \ HX_2 \ G \ HX_3 \ S$$

wherein

HX_1 is selected from the group consisting of N, Q, D, L, G and E,

HX_2 is selected from the group consisting of Y and T,

HX_3 is selected from the group consisting of V, I, F and L,

HCDR2 is of amino acid sequence which has the formula

$$W \ I \ HX_4 \ HX_5 \ HX_6 \ HX_7 \ G \ HX_8 \ T \ HX_9 \ Y \ HX_{10} \ HX_{11} \ HX_{12} \ F \ HX_{13} \ HX_{14}$$

wherein

HX_4 is selected from the group consisting of S, D, N, A, R, G and E,

HX_5 is selected from the group consisting of A, D, G, T, P, N and Y,

HX_6 is selected from the group consisting of N, D, L, A, P, T, S, I and R,

HX_7 is selected from the group consisting of N, S, T, D, G, K and I,

HX_8 is selected from the group consisting of D, T, E, Q, L, Y, N, V, A, M and G,

HX$_9$ is selected from the group consisting of N, I, L, Q, S, M, H, D and K,

HX$_{10}$ is selected from the group consisting of G and R,

HX$_{11}$ is selected from the group consisting of Q and R,

HX$_{12}$ is selected from the group consisting of E, K and G,

HX$_{13}$ is selected from the group consisting of Q and R,

HX$_{14}$ is selected from the group consisting of G and K,

HCDR3 is of amino acid sequence which has the formula

D HX$_{15}$ HX$_{16}$ HX$_{17}$ HX$_{18}$ W A R W HX$_{19}$ F HX$_{20}$ L

wherein

HX$_{15}$ is selected from the group consisting of S, R and D,

HX$_{16}$ is selected from the group consisting of S, N, D, T and P,

HX$_{17}$ is selected from the group consisting of S and R,

HX$_{18}$ is selected from the group consisting of S, N, A, I, R, P and K,

HX$_{19}$ is selected from the group consisting of F and Y,

HX$_{20}$ is selected from the group consisting of D and Y,

LCDR1 is of amino acid sequence which has the formula

G G LX$_1$ LX$_2$ LX$_3$ G LX$_4$ LX$_5$ L V H

wherein

LX$_1$ is selected from the group consisting of N, D and S,

LX$_2$ is selected from the group consisting of N, I, L, M, C, V, K, Y, F, R, T, S, A, H and G,

LX$_3$ is selected from the group consisting of I and V,

LX$_4$ is selected from the group consisting of S and G,

LX$_5$ is selected from the group consisting of K and R,

LCDR2 is of amino acid sequence which has the formula

D D G D R P LX_6

wherein

    LX_6 is selected from the group consisting of S and T,

LCDR3 is of amino acid sequence which has the formula

Q V W D T G S LX_7 P V LX_8

wherein

    LX_7 is selected from the group consisting of D and N,

    LX_8 is selected from the group consisting of V and I.

2. An isolated specific binding member according to claim 1, wherein

    HX_1 is selected from the group consisting of D and N,

    HX_2 is Y,

    HX_3 is L,

    HX_4 is selected from the group consisting of S and G,

    HX_5 is selected from the group consisting of T and A,

    HX_6 is N,

    HX_7 is selected from the group consisting of N and I,

    HX_8 is D,

    HX_9 is selected from the group consisting of N, D and K,

    HX_{10} is G,

    HX_{12} is selected from the group consisting of E and G,

    HX_{13} is Q,

    HX_{19} is F,

    LX_1 is selected from the group consisting of N and S,

LX_2 is selected from the group consisting of N, Y, T, S, and I,

LX_6 is S,

LX_7 is D.

5

3.   An isolated specific binding member according to claim 1, wherein

HX_1 is selected from the group consisting of N and D,

10   HX_2 is Y,

HX_3 is L,

HX_4 is selected from the group consisting of S and G,

HX_5 is selected from the group consisting of A and T,

HX_6 is N,

15   HX_7 is N,

HX_8 is selected from the group consisting of D and G,

HX_9 is selected from the group consisting of I, S, N and D,

HX_{11} is Q,

20   HX_{12} is E and K,

HX_{14} is G,

HX_{15} is S,

HX_{16} is selected from the group consisting of S and N,

HX_{17} is S,

25   HX_{18} is selected from the group consisting of S and N,

HX_{19} is F,

HX_{20} is D,

LX_1 is selected from the group consisting of N and D,

30   LX_3 is I,

LX_8 is V.

4.   An isolated specific binding member according to claim 1, wherein

HX$_7$ is selected from the group consisting of N, S, T, D, G and K,

HX$_8$ is selected from the group consisting of D, T, E, Q, L, Y, N, V, A, M,

HX$_9$ is selected from the group consisting of N, I, L, Q, S, M and H,

HX$_{10}$ is G,

HX$_{11}$ is Q,

HX$_{12}$ is F,

HX$_{13}$ is Q,

HX$_{14}$ is G,

HX$_{15}$ is S,

HX$_{16}$ is selected from the group consisting of N and S,

HX$_{17}$ is S,

HX$_{18}$ is selected from the group consisting of N and S,

HX$_{19}$ is F,

HX$_{20}$ is D,

LX$_1$ is N,

LX$_2$ is selected from the group consisting of N and I,

LX$_3$ is I,

LX$_4$ is S,

LX$_5$ is K,

LX$_6$ is S,

LX$_7$ is D,

LX$_8$ is V.

5.   An isolated specific binding member according to claim 4, wherein

HX$_1$ is selected from the group consisting of N, Q and D,

HX$_3$ is selected from the group consisting of L, V and I,

HX$_4$ is selected from the group consisting of S, N, A and R,

HX$_5$ is selected from the group consisting of A, D, T, G, N and Y,

5   HX$_6$ is selected from the group consisting of N, A, P, S, D and I,

HX$_7$ is selected from the group consisting of N, T, D and G,

HX$_8$ is selected from the group consisting of D, Q, Y and
10 N,

HX$_9$ is selected from the group consisting of N, Q, S and I.

6. A specific binding member according to any one of claims 1
15 to 5 that neutralizes human IL-13.

7. A specific binding member according to claim 6 that
neutralizes human IL-13, with a potency equal to or better
than the potency of a IL-13 antigen-binding site formed by the
20 BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain
(SEQ ID NO: 16), the potency of the specific binding member
and the potency of the antigen-binding site being as
determined under the same conditions.

25 8. An isolated specific binding member according to any one
of claims 1 to 7 which binds a human IL-13 variant in which
arginine at position 130 is replaced by glutamine.

9. An isolated specific binding member according to any one
30 of claims 1 to 7 which binds non-human primate IL-13.

10. An isolated specific binding member for human IL-13,
comprising an antibody antigen-binding site which is composed
of a human antibody VH domain and a human antibody VL domain

and which comprises a set of CDR's HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the set of CDR's consists of a set of CDR's selected

5   from the group consisting of:

the BAK278D6 set of CDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid

10 sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6,

a set of CDR's which contains one or two amino acid substitutions compared with the BAK278D6 set of CDR's, and

15   each set of CDR's as shown for individual clones in Table 1.

11.   An isolated specific binding member according to claim 10 wherein the one or two substitutions are at one or two of the

20 following residues within the CDRs, using the standard numbering of Kabat.

31, 32, 34 in HCDR1

25 52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2

96, 97, 98, 99, 101 in HCDR3

26, 27, 28, 30, 31 in LCDR1

30

56 in LCDR2

95A, 97 in LCDR3

12. An isolated specific binding member according to claim 11 wherein the one or two substitutions are made at the following positions from among the identified groups of possible substitute residues for each position:

5

<u>Position of</u>   <u>Substitute Residue</u>
<u>substitution</u>   <u>selected from the group</u>
               <u>consisting of</u>

Position of substitution	Substitute Residue selected from the group consisting of
10	31 in HCDR1:	Q, D, L, G and E
	32 in HCDR1:	T
	34 in HCDR1:	V, I and F
15	52 in HCDR2:	D, N, A, R, G and E
	52A in HCDR2:	D, G, T, P, N and Y
	53 in HCDR2:	D, L, A, P, T, S, I and R
	54 in HCDR2:	S, T, D, G, K and I
	56 in HCDR2:	T, E, Q, L, Y, N, V, A, M and G
20	58 in HCDR2:	I, L, Q, S, M, H, D and K
	60 in HCDR2:	R
	61 in HCDR2:	R
	62 in HCDR2:	K and G
	64 in HCDR2:	R
25	65 in HCDR2:	K
	96 in HCDR3:	R and D
	97 in HCDR3:	N, D, T and P
	98 in HCDR3:	R
30	99 in HCDR3:	S, A, I, R, P and K
	101 in HCDR3:	Y
	26 in LCDR1:	D and S
	27 in LCDR1:	I, L, M, C, V, K, Y, F, R, T, S, A, H and G

```
      28 in LCDR1:     V
      30 in LCDR1:     G
      31 in LCDR1:     R


5     56 in LCDR2:     T


      95A in LCDR3:    N
      97 in LCDR3:     I.
```

10   13.   An isolated specific binding member according to claim 12
     wherein there are two substitutions compared with the BAK278D6
     set of CDR's, at HCDR3 residue 99 and LCDR1 residue 27.

     14.   An isolated specific binding member according to claim 13
15   comprising the BAK278D6 set of CDR's with a substitution at
     HCDR3 residue 99 selected from the group consisting of S, A,
     I, R, P and K, and/or a substitution at LCDR1 residue 27
     selected from the group consisting of I, L, M, C, V, K, Y, F,
     R, T, S, A, H and G.

20
     15.   An isolated specific binding member according to claim 13
     comprising the BAK278D6 set of CDR's with S substituted for N
     at HCDR3 residue 99 and/or I substituted for N. at LCDR 1
     residue 27.

25
     16.   An isolated specific binding member according to any one
     of claims 10 to 15 wherein HCDR1, HCDR2 and HCDR3 of the VH
     domain are within a germ-line framework and/or LCDR1, LCDR2
     and LCDR3 of the VL domain are within a germ-line framework.

30
     17.   An isolated specific binding member according to any one
     of claims 10 to 16 which binds a human IL-13 variant in which
     arginine at position 130 is replaced by glutamine.

18. An isolated specific binding member according to any one of claims 10 to 17 which binds non-human primate IL-13.

19. A specific binding member according to any one of claims
5   16 to 18 comprising the BAK502G9 VH domain (SEQ ID NO: 15).

20. A specific binding member according to any one of claims 16 to 19 comprising the BAK502G9 VL domain (SEQ ID NO: 16).

10 21. A specific binding member according to any one of claims 10 to 20 that binds IL-13 with affinity equal to or better than the affinity of an IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain (SEQ ID NO: 16), the affinity of the specific binding
15   member and the affinity of the antigen-binding site being as determined under the same conditions.

22. A specific binding member according to any one of claims 10 to 21 that neutralizes human IL-13.
20

23. A specific binding member according to claim 22 that neutralizes human IL-13, with a potency equal to or better than the potency of a IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain
25   (SEQ ID NO: 16), the potency of the specific binding member and the potency of the antigen-binding site being as determined under the same conditions.

Figure 1

- ■ BAK167A11 scFv
- □ BAK615E3 scFv
- △ Irrelevant scFv

Figure 2

- ■ BAK278D6 scFv
- □ BAK502G9 scFv
- △ Irrelevant scFv

Figure 3

Figure 4A

**Inhibition of human·IL-13
dependent TF-1 cell proliferation**

■ BAK278D6 scFv
△ Irrelevant scFv

Figure 4B

**Inhibition of human IL-13 variant (Q130R)
dependent TF-1 cell proliferation**

■ BAK278D6 scFv
△ Irrelevant scFv

Figure 4C

**Inhibition of non-human primate IL-13
dependent TF-1 cell proliferation**

■ BAK278D6 scFv
△ Irrelevant scFv

Figure 5

*p<0.05
**p<0.01
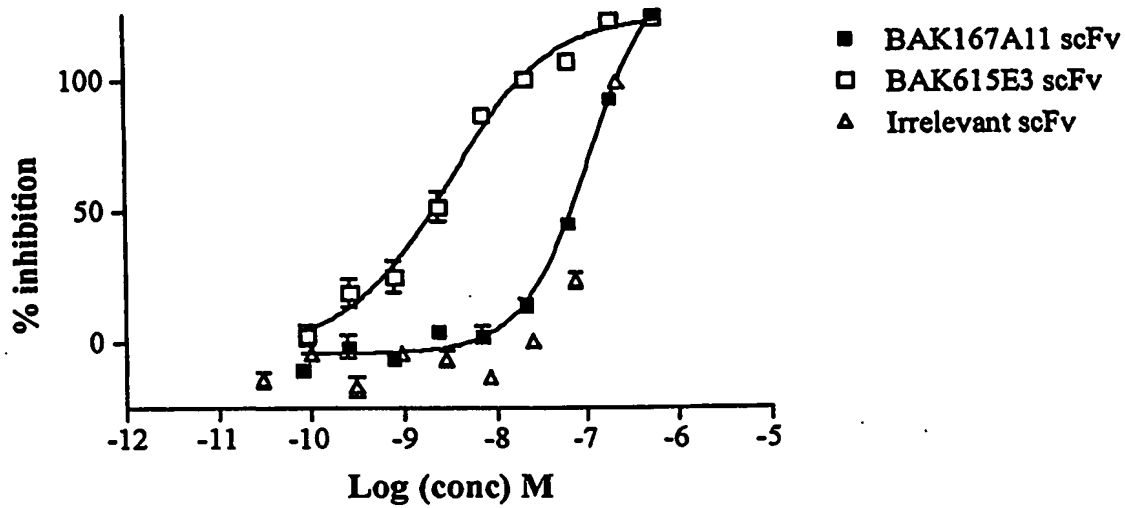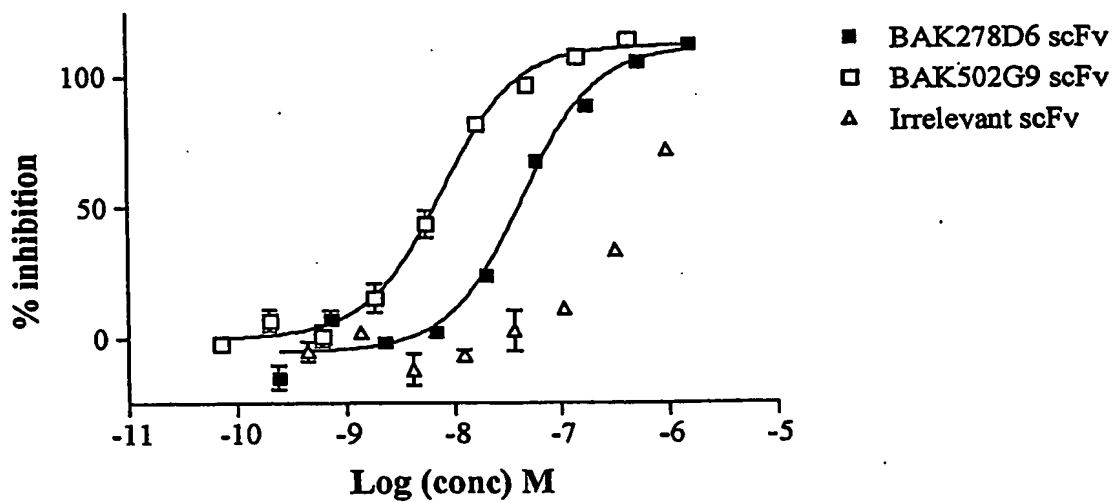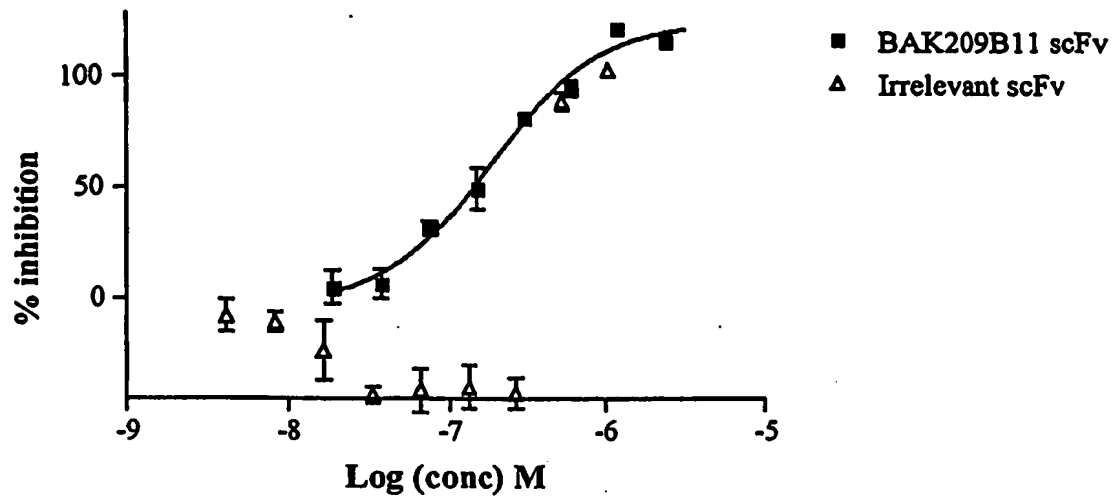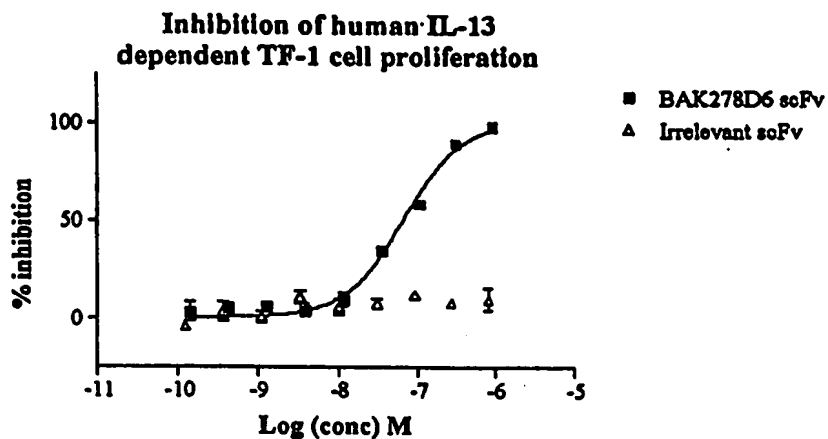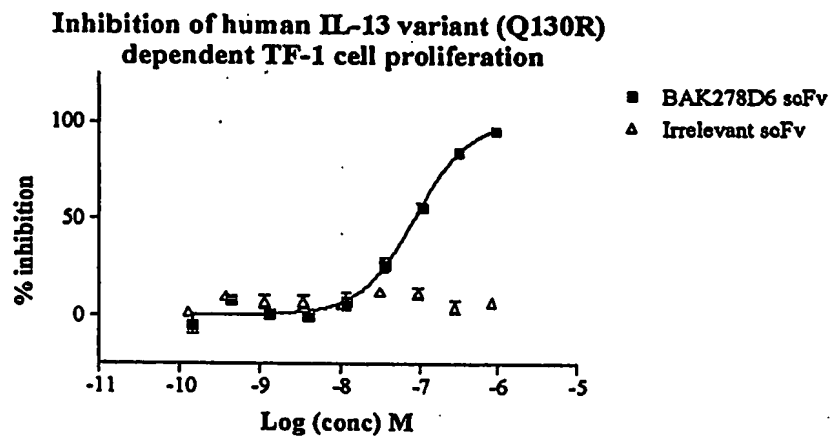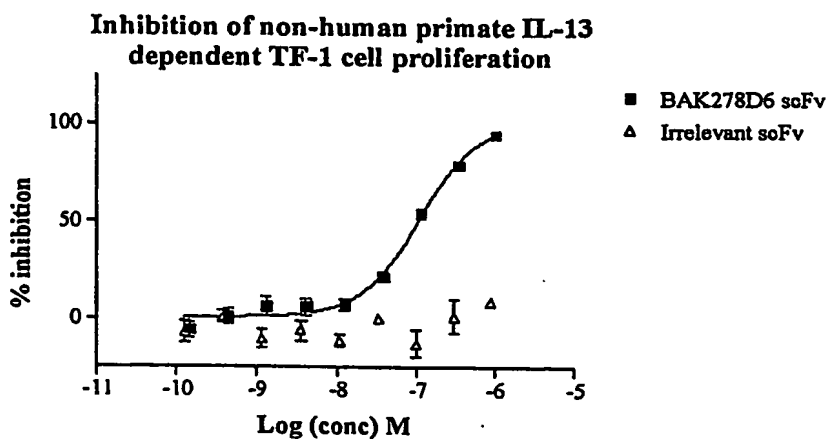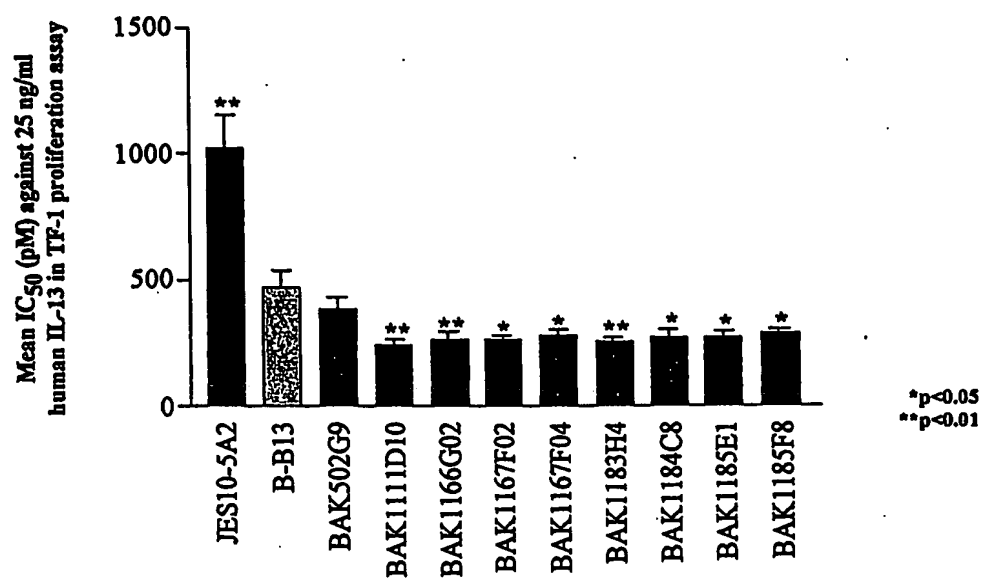
Figure 6A

**Inhibition of human IL-13 FLAG
dependent TF-1 cell proliferation**

- ■ BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4
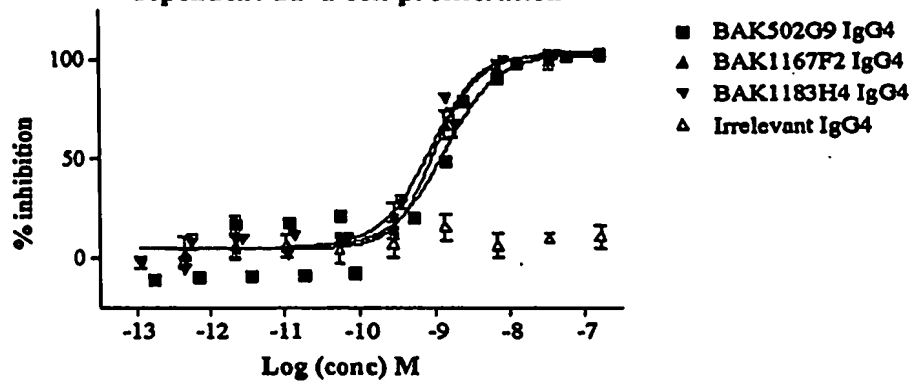
Figure 6B

**Inhibition of IL-13 (Q130R) FLAG
dependent TF-1 cell proliferation**

- ■ BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4
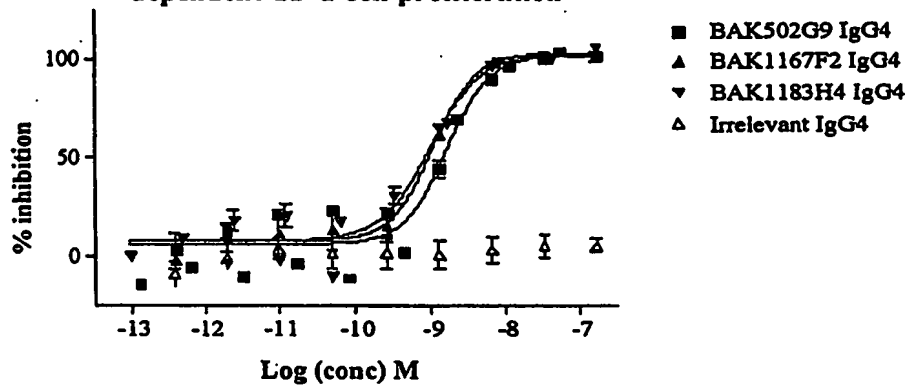
Figure 6C

**Inhibition of non-human primate IL-13 (FLAG-
tagged) dependent TF-1 cell proliferation**

- ■ BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4
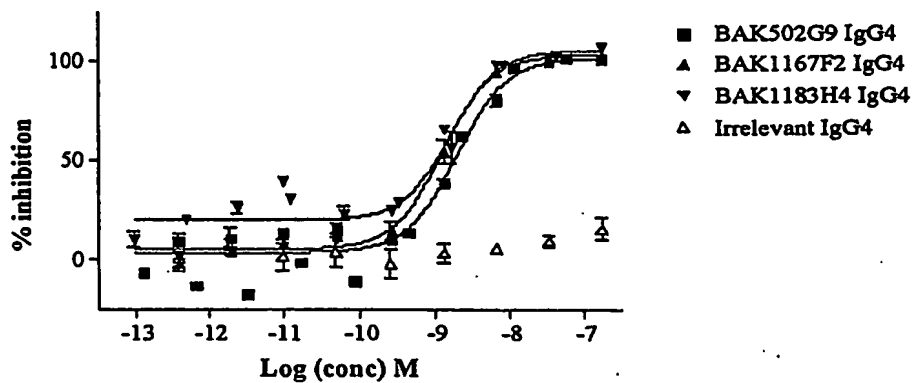
Figure 7

Figure 8

Figure 9

- ■ BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4

Figure 10A

- ■ BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4

Figure 10B

- ■ BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4

Figure 11

- ■ BAK209B11 IgG4
- △ Irrelevant IgG4

Figure 12

**p < 0.01

Figure 13

*p<0.05
**p<0.01

Treatment

Figure 14

*p<0.05
**p<0.01

Treatment

Figure 15

Treatment

*p<0.05
**p<0.01

Figure 16

Treatment

Figure 17

Number of monocytes/macrophages (x10⁴)

Treatment

BAK209B11

Non-sensitised / Sensitised / 12µg / 36µg / 119µg / 357µg / 357µg Irrelevant IgG4

Figure 18

Number of leukocytes (x10⁴)

Treatment

JES10-5A2

CMC / CMC + 2µg IL-13 / 15µg / 150µg / 150µg Irrelevant rat IgG1

*p<0.05
**p<0.01

Figure 19

```
              10          20          30          40
Human IL-13   MALLLTTVIALTCLGGFASP[G]PVPPSTAL[R]ELIEELVNIT
Cynomolgus IL-13  MALLLTTVIALTCLGGFASP[S]PVPPSTAL[K]ELIEELVNIT
              MALLLTTVIALTCLGGFASP PVPPSTAL ELIEELVNIT


              50          60          70          80
Human IL-13   QNQKAPLCNGSMVWSINLTAG[M]YCAALESLINVSGCSAIE
Cynomolgus IL-13  QNQKAPLCNGSMVWSINLTAG[V]YCAALESLINVSGCSAIE
              QNQKAPLCNGSMVWSINLTAG YCAALESLINVSGCSAIE


              90          100         110         120
Human IL-13   KTQRML[S]GFCPHKVSAGQFSSL[H]VRDTKIEVAQFVKDLL[L]
Cynomolgus IL-13  KTQRML[N]GFCPHKVSAGQFSSL[R]VRDTKIEVAQFVKDLL[V]
              KTQRML GFCPHKVSAGQFSSL VRDTKIEVAQFVKDLL


              130
Human IL-13   HLKKLFREG[R]FN
Cynomolgus IL-13  HLKKLFREG[Q]FN
              HLKKLFREG FN
```

Figure 20

Effect of a single 10mg/kg dose of BAK502G9 (IgG4) on serum IgE levels in allergic but non-challenged cynomolgus monkeys
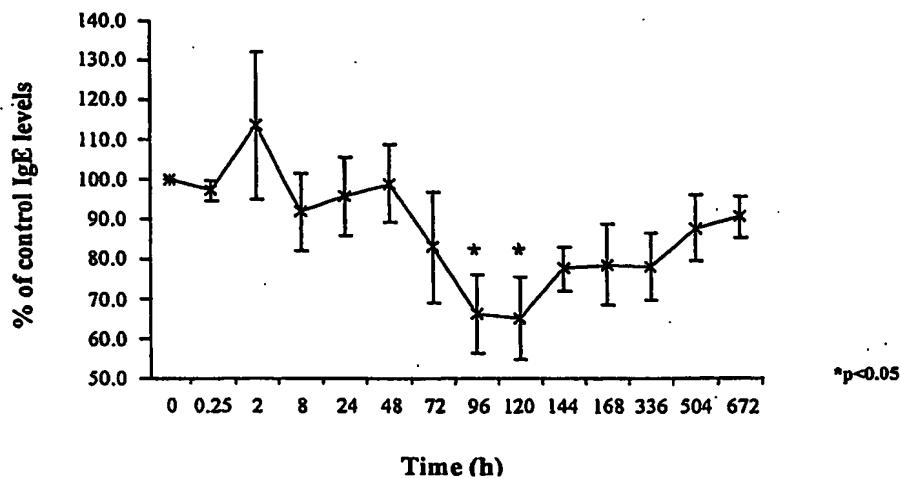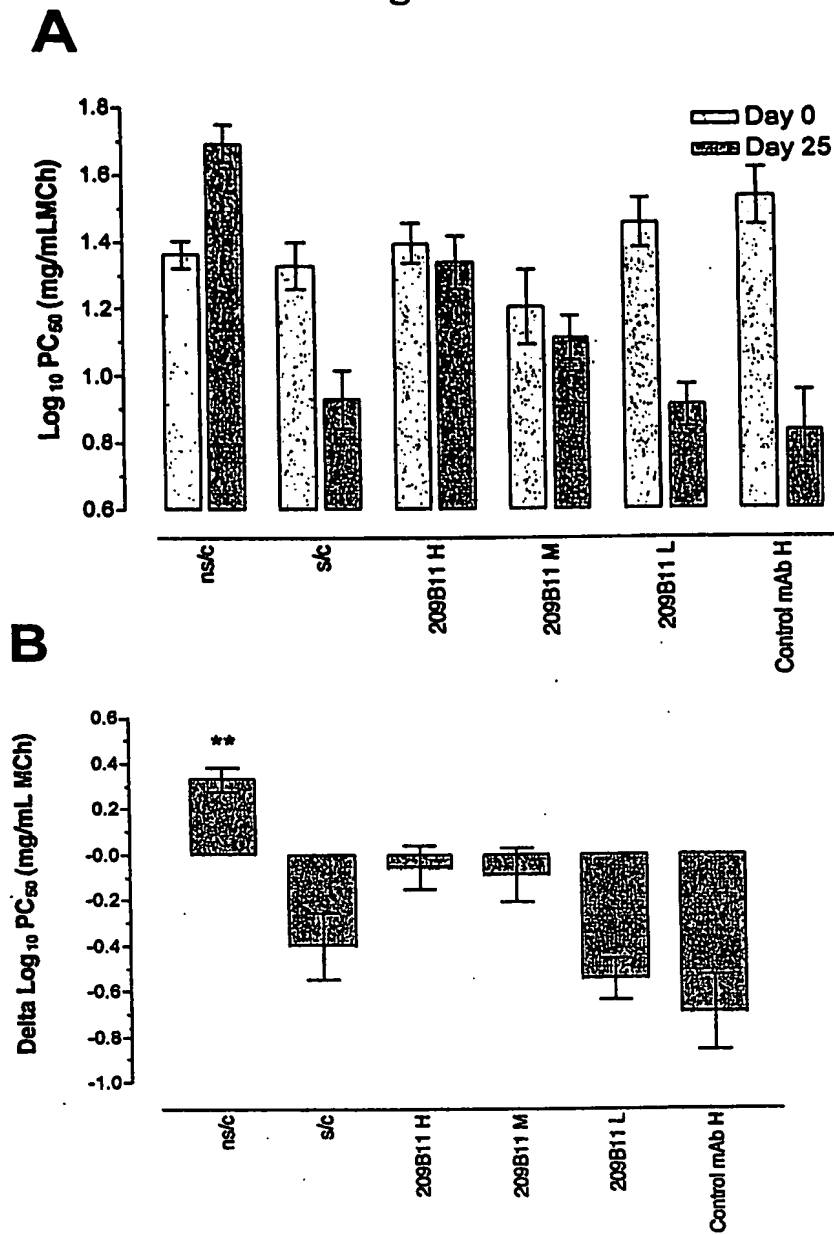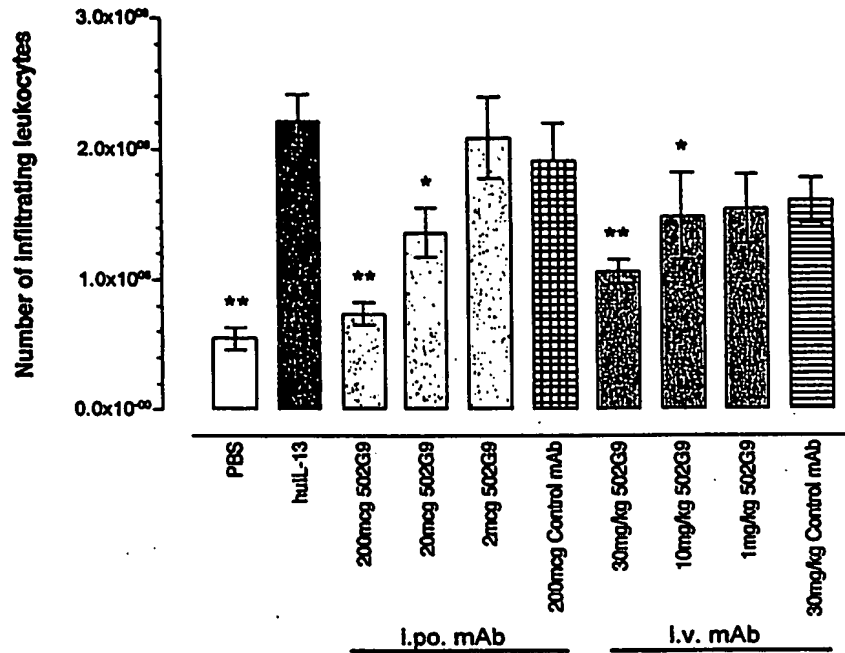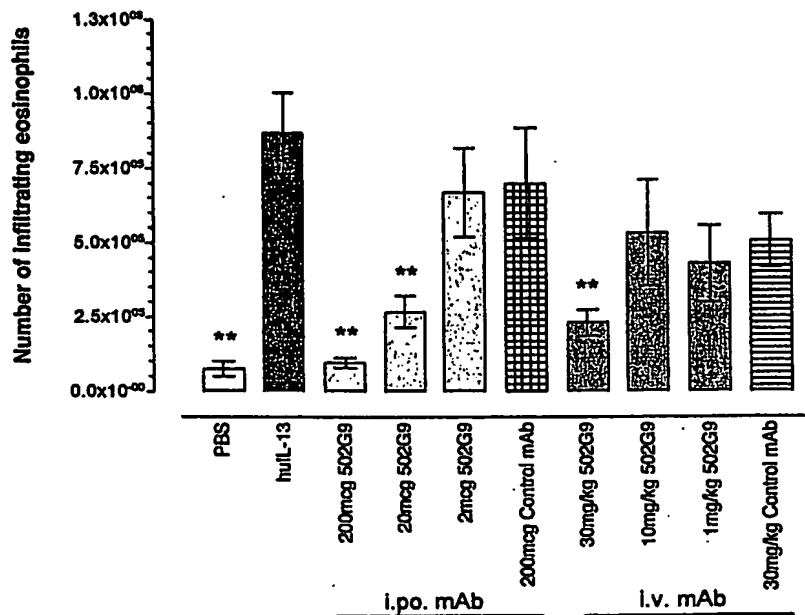

$*p<0.05$

Time (h)

Figure 21

A

B

** p<0.01 vs s/c control; One-way ANOVA followed by
Dunnett's multiple comparisons test

Figure 22

A

B

A and B; *p<0.05, ** p<0.01 vs huIL-13 control; One-way ANOVA on log-transformed data, followed by Dunnett's multiple comparisons test.